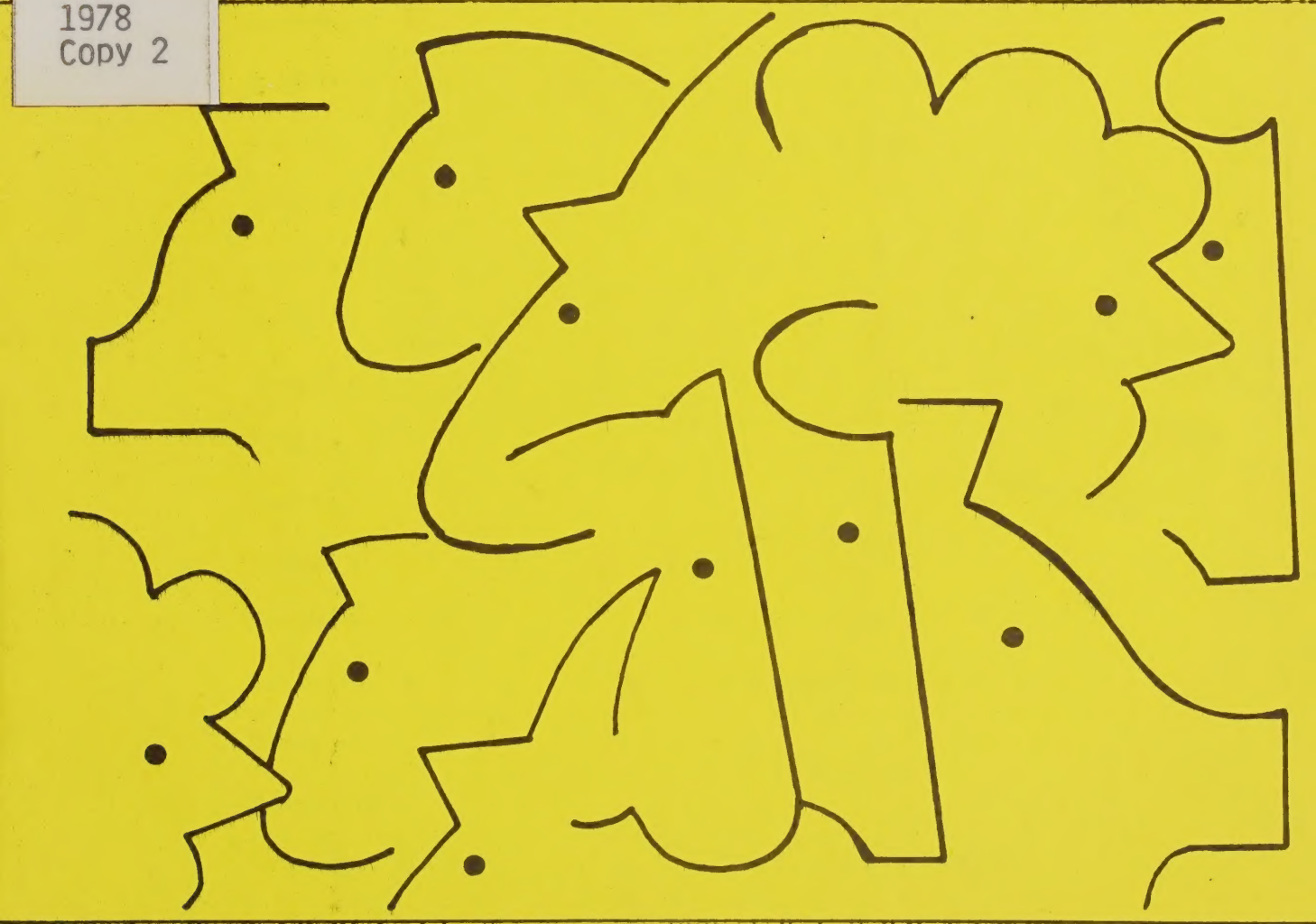
REFERENCE MANUAL

FOREIGN ANIMAL DISEASE COURSES

PLUM ISLAND ANIMAL DISEASE CENTER



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REFERENCE MANUAL
FOREIGN ANIMAL DISEASE COURSES
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REFERENCE MANUAL

FOREIGN ANIMAL DISEASE COURSES

FORWARD

This reference manual provides up-to-date background information on those foreign animal diseases which are demonstrated in PIADC courses. The major objective of these courses is to give field diagnosticians experience in the clinical aspects of selected exotic diseases (as well as a few similar domestic diseases). Most of your time will be spent participating in actual disease exercises and demonstrations. During the short time you are at PIADC you may not have time to avail yourself of our excellent library or to read lengthy reviews. There is a vast and scattered literature on many diseases, such as foot-and-mouth disease; on others, such as swine vesicular disease, little has been written and the few papers that exist might be difficult for you to look up in the field.

The reviews of each disease have been prepared and reviewed by the PIADC faculty to provide you with succint and cogent data on the diseases you will meet in the laboratory.

You may find the Reference Manual of value later, also. For field use PIADC has prepared a Pocket Handbook on diseases exotic to most of the Americas. This book has been scaled down to pocket size, but is so designed that it still contains a great deal of memory jogging information, including more than 150 color illustrations of the main clinical features of 10 major exotic diseases. These may be readily studied in a microfiche reader, with a 10X hand lens or even projected by the TAYLOR-MERCHANT microfice projector* and similar inexpensive devices. The color illustrations and the short descriptions may be valuable in emergency situations.

If lesions other than those illustrated are seen in the demonstrations or in the field, the Pocket Handbook will be updated to include these in the future. As new data are accumulated both the Reference Manual and Pocket Handbook will be revised and the new editions made available.

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*Taylor-Merchant Corporation, New York, N.Y. 10036. This is for information only and does not imply endorsement.

There are numerous other diseases foreign to the Americas which cannot be included in the courses given at PIADC within the time alloted. You are urged to obtain a copy of FOREIGN ANIMAL DISEASES, Their Prevention, Diagnosis and Control (The "Gray Book") by the Committee on Foreign Animal Diseases of the United States Animal Health Association. The latest edition was printed in 1976. (U.S. Animal Health Association, 1444 East Main St., Richmond, Va. 23219)

If your interests and responsibilities lie in the control and regulatory field you should consult the EMERGENCY ANIMAL DISEASE ERADICATION GUIDE (The "Red Book") from the Animal and Plant Health Inspection Service (APHIS). This book was reprinted in 1976; a new edition will be ready soon.

You may have still other interests. In each class there have been those interested in some aspect of research or the laboratory tests used to make or confirm diagnoses. We have several microfiche giving detailed protocols for several tests and others on electron microscopy related to animal viruses; others show clinical aspects of various diseases. In addition, motion pictures and slide sets are available. You are invited to write to the Director at any time concerning your interests and needs related to emergency diseases.

AFRICAN HORSE SICKNESS *

I. Identification of disease:

A. Definition.--African horse sickness (AHS), also termed perdesietke and pestis equorum, is an insect-borne disease of equidea, caused by a virus of 9 immunological types. It occurs as an acute or subacute disease, and is highly fatal in a susceptible equine population.

B. Etiology.--The virus particle is small, measuring 70-80 nm in diameter and is estimated to have 92 rod-shaped subunits radiating from a spherical body. The virulent virus can be maintained in OGC solution at 4° C for several years, but it is destroyed in 2 weeks at 37° C. The virus is destroyed by treatment with acetic acid (pH 3 and lower), and its infectivity is destroyed in 5 minutes at 70° C.

The virus was adapted to baby mice by intracerebral inoculation. Mortality in mice increases upon further passages, with a subsequent decrease in virulence of the virus for horses. Although the virus loses its pathogenicity for equidae, it does not lose its antigenic and immunizing capabilities for these species.

C. History.--The disease was endemic in Africa and apparently caused great mortality in equidae which were brought by the early settlers. Early research with the virus was slow until Alexander (1933-1935) adapted the virus to mice by intracerebral passage. The disease was restricted to Africa until it made its appearance in Middle Eastern countries. In 1966 it spread to North Africa and the southern tip of Spain. The disease there was eradicated by 1969 as a result of vaccination and other measures.

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*Prepared by A. H. Dardiri

II. Signs:

A. Clinical features.--To the clinician observing an early case of AHS, the most obvious signs are depression and high fever. However, the signs of the disease vary, depending on the form in which it manifests itself.

The pulmonary form is acute and may be of quite sudden onset, marked by respiratory involvement and fever. Coughing spasms occur, and large quantities of frothy fluid may be discharged from the nostrils. The head and neck are extended, the ears droop, and severe sweating occurs. Finally, the animal lies down, nearly choking. Until the terminal stages, the animal appears hungry and attempts to eat. This form of the disease is highly fatal.

The cardiac form is characterized by fever and a slower course. Swelling of the head, neck, and chest are typical manifestations of this form. Edema of the supraorbital fossea, eye lids, and lips is common. Recovery is more common with this form.

A mixture of the pulmonary and cardiac forms may also occur, with either one predominating initially; the mixed forms are less readily diagnosed until necropsy examination is made.

Mild cases may also occur with the only indications being a rise in temperature, rapid pulse, and difficult breathing. This form occurs most often with the less virulent viruses or where some degree of immunity is present. Slight symptoms of this type may be seen following vaccination.

B. Incubation period.--The incubation period is less than 9 days under natural conditions. Experimentally, the incubation period varies from 2-21 days. Usually it is about 14 days.

III. Pathologic changes:

A. Postmortem lesions.--The lesions vary in accordance with the form of disease. With the pulmonary form, the most conspicuous lesions are edema of the lungs and hydrothorax, the lungs remaining fully distended. The subpleural and interlobular tissues are heavily infiltrated with yellowish gelatinous exudate. The bronchi, trachea, pharynx, and nostrils are filled with froth and fluids. The stomach contains a viscid mucous mixed with food. Also, the mucosa of the fundus is reddened and edematous.

In the cardiac form, the outstanding lesions are gelatinous exudates in the subcutaneous, subfacial, and intramuscular tissues and lymph nodes. A massive hydropericardium is always seen and hemorrhages and ecchymosis are found on the epicardial or endocardial surfaces, or bordering the coronary vessels.

As in the pulmonary form, acites may be found, and the mesentery glands may be swollen. Edema of the lungs is slight. Stomach lesions are the same as in the pulmonary form.

In the mixed form of AHS, lesions common to both the lung and cardiac forms are found.

B. Microlesions.--The histopathological changes in the equine organs are the result of increased permeability of the capillary walls and impairment of the circulatory system.

The lungs show serous infiltration of the intralobular tissue with distention of the alveoli and congestion of the capillaries.

The central veins of the liver are distended, the interacinous tissue contains erythrocytes and blood pigment, while the parenchymatous cells exhibit fatty degeneration.

Various degrees of round cell infiltration can be seen in the cortex of the kidneys.

The spleen is congested and shows hemorrhagic extravassation into the pulp.

Variable degrees of congestion are present in the gastric and intestinal mucosae, and cloudy swelling in the myocardial and skeletal muscles.

IV. Diagnosis:

A. In the field.--In enzootic areas typical clinical features characteristic of the disease, such as dyspnea, edema of the supraorbital fossea, subcutaneous edema of the head and neck, respiratory and heart lesions, excess of pericardial and pleural fluids and severe gastritis, aid in forming a presumptive diagnosis.

B. Laboratory diagnosis.--Specimens required for laboratory examination in the study of AHS are:

1. Blood for virus isolation. Blood should be collected, with equal amounts of OGC fluid and kept refrigerated.

2. Tissues for virus isolation. Spleen and lymph nodes are suitable for virus isolation and may be collected in 50% neutral glycerin in buffered saline.

3. Serum for serological tests. Two or 3 serum samples are required; one to be collected at onset of disease; the second, one week after the decline of fever; and a third, 14 to 21 days following temperature peak.

Confirmation of the disease in the laboratory is arrived at by isolation of the AHS virus, using 3- to 6-day-old mice. The virus is then assayed in mice or cell culture and then identified by serological tests.

The most common and specific tests are the complement-fixation (CF) and virus neutralization (VN) tests.

Complement-fixing antibodies are of short duration and as the test is not AHS virus type-specific, it is invaluable in the diagnosis of the disease.

Virus-neutralizing antibodies appear shortly after the CF antibodies and persist for a prolonged period. The VN test also is invaluable for typing the AHS viruses.

C. Differential diagnosis*.--Although the epizootiological and clinical signs of African horse sickness may lead to suspicion of infection with the disease, clinical features and postmortem appearance of animals affected by the disease can be confused with other diseases to which equidae are susceptible, such as viral arteritis and equine infectious anemia.

1. Viral arteritis. Manifestations in animals which have been infected with viral arteritis are conjunctivitis, palpebral edema, and edema of the legs, abdomen, mammary glands, scrotum, and sheath. In this disease, the gross lesions may include petechial hemorrhages on the serous surfaces, and edema. Yellow, gelatinous infiltrations are observed in the mediastinal tissues, mesentery, and sublumbar tissues also.

Virological and histopathological examinations are required for differential diagnosis.

This disease is transmitted by contact, and it is not fatal to suckling mice.

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*See also appendices A and B.

2. Equine infectious anemia (EIA). The acute disease is characterized by sudden onset and a rise in temperature--usually to about 105 to 108° F. The affected animal may show continuous fever or frequent intermittent attacks. The characteristic epizootiology of EIA may lead to diagnosis of this disease. Infectious anemia is suspected when disease breaks out among several horses in a group brought together from widely scattered areas for racing, horse shows, country fairs, and rodeos. A few horses may die at the beginning of the outbreak, but it is usually followed by the subacute form and subsequent recovery. The particular nature of the petechial and ecchymotic types of hemorrhages on the surfaces of the major parenchymatous organs and serous and mucous membranes are distinguishing features.

V. Prognosis:

In susceptible equine populations the fatalities range between 80-90%. In enzootic areas, the mortality rate is modified in proportion to the resistance acquired by the equine population as a result of previous exposure or natural resistance.

VI. Epizootiology:

A. Geographical distribution.--African horse sickness was confined to certain regions in Africa until the summer of 1959 when it appeared in Iran, West Pakistan, and nearby Afghanistan. Instead of dying out over the winter as expected, it appeared in the spring of 1960 and rapidly spread to India, Turkey, Cyprus, Iraq, Syria, Lebanon, and Jordon. Thus, it appears that the disease can exist permanently in any area of the world where climatic conditions favor survival of culicoides, the principal insect vectors, throughout the winter months.

The role of climatic conditions seemed important in the occurrence of new outbreaks and the spread of the disease. It was observed in the 1966 disease epizootic in Tunesia, Algeria, Morocco, and Spain that many outbreaks of AHS arose after rainfall which was followed by 2 weeks of temperate, dry weather. This favored the rapid multiplication of insects.

B. Transmission.--Although various insects have been incriminated, culicoides are the chief vectors for the transmission of African horse sickness. However, there is insufficient information as to the chief reservoir of the virus. It appears that when the virus is maintained in an invertebrate, with optimal climatic conditions and a susceptible equine population, the disease makes its appearance. Equidae appear to be dead-end hosts although unvaccinated, susceptible animals could serve to amplify and enhance viral activity, especially in the presence of large numbers of easily infected culicoides.

C. Host range.--Equidea were found to be naturally susceptible, with horses and ponies the most susceptible. The Middle Eastern donkey has been found much less susceptible than the mule, but more susceptible than the African donkey which is quite resistant.

Dogs exhibit transitory viremia following ingestion of large quantities of meat and blood from infected equines.

VII. Control and eradication:

A. Preventive measures.--Although chronic carriers in AHS are not known, importation of equidae from countries in which the disease is enzootic to those free from it must be restricted. If importation is allowed the animals should be quarantined 30-60 days. Animals in quarantine must be maintained free from external parasites and protected from biting insects.

B. Natural immunity.--Equidae recovered from the disease develop solid immunity against the homologous strain of virus but may be susceptible to infection by other virus strains. Foals from immune dams have a natural immunity which protects them for the first few months of life and cannot be successfully vaccinated until their maternal immunity has waned at about the 8th month.

C. Induced immunity.--McIntosh reported 42 immunologically distinct strains of virus which could be divided into 9 virus types. The work of Alexander has made possible the production of an attenuated virus vaccine. A vaccine is produced by serial intracerebral propagation of the virus in mice. At the 100th serial passage, the virus is sufficiently attenuated to produce effective vaccine. The virus becomes neurotropic to mice and loses its virulence for the horse.

A polyvalent vaccine of 6 virus types has been used successfully to protect susceptible animals in South Africa.

More recently, the neurotropic mouse-adapted virus was propagated in cell culture and used in the 1966 disease outbreak in Tunesia, Algeria, Morocco, and Spain.

African horse sickness vaccine inactivated by formalin has been tried, giving protection to susceptible horses. The potential of such a preparation has not been fully evaluated in the field to determine its limitations and advantage.

There is always concern regarding a possible return of virulence during serial passage of the neurotropic virus in equines and many countries that are free from the disease would not accept a live vaccine as a prophylaxis.

The extensive spread of the disease in the Middle East and North Africa attests to the problems involved in its confinement, and confirms the necessity for constant vigilance to prevent the introduction of animals from infected areas into clean areas.

VIII. Public health aspects:

The disease is not transmissible to man.

Selected References:

1. Merchant, I. A. and Barner, R. D. 1964. African Horse Sickness. In "Infectious Diseases of Domestic Animals." Iowa State University Press, Ames, Iowa. pp. 417-421.

2. Maurer, F. D. and McCully, R. M. 1963. African Horse Sickness. Am. J. Vet. Res. 24:235-266.

APPENDIX A

DIFFERENTIAL DIAGNOSIS OF AFRICAN HORSE SICKNESS (AHS)

DISEASE FEATURE	DISEASES				
	AHS	EVA*	EIA**	TRYPS.	PIROP.
Clinical					
subc. edema	head, ventral body surface	ventral body surface tendon sheaths	abdomen & legs	generally distributed	
	++++	+++	+++	++	
Infra-orbital forces	++++				rare & late
PM lesions					
pulmonary edema	++++	++			rare & late
bronchopneumonia	+	+++			
hydrothorax	+++	++			
edema of pharynx	++++	++			
edema of intestines	+++	++			
edema general tissue	yellow & gelat. ++++	yellow & gelat. ++	watery +		
hemorrhages	++++		+++		
ictrus			++		
Epidemiology					
seasonal	+++		+++		+++
recurrent illness			+++	+++	+++
Microscopic exam					
arteries		+++			
bone marrow			+++		++
blood smears				+++	+++

*Equine Viral Arteritis

**Equine Infectious Anemia

APPENDIX B

SPECIMENS AND APPROACH FOR LABORATORY DIAGNOSIS OF EQUINE DISEASES WHICH MAY BE CONFUSED WITH AFRICAN HORSE SICKNESS

Diagnosis Suspected	Source of Material		Laboratory examination	
	Clinical	Postmortem	Antigen (virus)	Antibody (convalescent serum)
African horse sickness	Blood	Spleen	Mouse inoculation Equine transmission Cell culture	CF test, Mouse inoc. Tissue culture VN test, FA, AGDP
Equine infectious anemia	Blood and serum	Spleen	Equine transmission	AGDP test
Viral arteritis	Nasal swabs	Spleen	Cell culture Equine transmission Histopathology	VN test - tissue culture
Babesiosis	Blood smears	Spleen	Microscopic	
Anthrax	Blood and tissue	Smears, swabs, tissues	Microscopic culture Animal inoculation FA test	
Venezuelan equine encephalomyelitis	Blood, serums	Brain	Laboratory animals, cell cultures, Histopathology	VN test

AFRICAN SWINE FEVER *

I. Identification of disease:

A. Definition.--In its most usual and distinctive form African swine fever (ASP) is a highly contagious, peracute, febrile, and septicemic viral disease of domestic swine that is characterized by marked hemorrhages in the internal organs, cyanosis of the skin, and mortality closely approaching 100%. However, in areas where the disease has become enzootic in domestic swine, the mortality may be somewhat reduced and an increased frequency of subacute and chronic infections may be encountered. In any case, laboratory tests are usually required to establish positive differentiation between African swine fever and hog cholera (HC).

B. Etiology.--The virus of ASF (ASFV) is an icosahedron 175 to 215 nm in diameter and has an outer envelope acquired from the cytoplasmic membrane of the host cell. It is sensitive to lipid solvents and contains deoxyribonucleic acid (DNA). It is neither morphologically nor immunologically related to the virus of HC, nor is it related to any other mammalian virus presently known. It replicates entirely within the cytoplasm of the host cell. It is the only so-called icosahedral cytoplasmic DNA virus known to infect mammals. Other viruses of this type are found in amphibians, fish, insects, and plants.

In Africa, ASFV has been found in three species of indigenous wild pigs. They serve as reservoirs of the virus and apparently tolerate the infection without suffering ill effects. Yet, when this virus is transmitted to domestic swine, it usually gives rise to the lethal, peracute form of the disease.

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*Prepared by W.R. Hess, I.C. Pan and C. J. DeBoer

The Argasid ticks, Ornithodoros erraticus in Spain and Ornithodoros moubata porcinus in Africa, are capable of transmitting ASFV. The virus is known to persist for at least a year in the ticks and virus replication and transovarial infection have been demonstrated in them. They must, therefore, be regarded as reservoirs as well as vectors of the virus.

The serums of chronically infected pigs, or pigs that have survived infections with laboratory-modified strains of ASFV, usually contain antibodies capable of forming precipitates with a number of ASFV-associated antigens. Although virus neutralizing antibodies have not been convincingly demonstrated, these animals are often refractory to large challenge doses of the fully virulent homologous virus isolate. However, when challenged with a virus isolate from a different geographical location, the animal may die with the typical acute disease. It is therefore thought that several immunologic types of the virus may exist.

C. History.--The disease was first described by Montgomery who studied it extensively in Kenya during the period from 1910 to 1915 when outbreaks involving 1366 swine occurred with a mortality of 98.9%. He established its viral nature, determined it to be immunologically distinct from HC, studied the survival of the virus under a variety of environmental conditions, explored methods of transmission and immunization, studied its host range and suggested the possible role of wild swine in maintaining the disease in nature.

In the years from 1915 to 1957, outbreaks of ASF occurred in a number of areas south of the Sahara in Africa, and the recognition of the disease's potential threat to the world's swine industry began to emerge. During this period Montgomery's findings were confirmed. The wart hog and

bush pig were found to be inapparent carriers of the virus in Africa, but the mode of transmission from the wild to domestic pig remained obscure. Neutralizing antibodies were not found and though all available adjuvants and inactivation methods were tried, a suitable vaccine was not developed.

The USA was apparently the first major swine-raising country to fully recognize the destructive potential of ASF and assigned investigators to study the disease in Kenya in the early 1950's. However, it was not until the disease appeared in Portugal in 1957 and again in 1960 and then spread into Spain that it aroused the concern that it rightly deserved. Fortunately, workers in Kenya developed diagnostic procedures that greatly enhanced efforts to control and eradicate the disease, and for a few years it was successfully confined to the Iberian peninsula. But it had become established as an enzootic disease in Spain and Portugal, and perhaps through continued residence in domestic swine and the disasterous application of so-called modified live vaccines, the disease picture began to change. Subacute and chronic infections became more prevalent and its similarity to HC was even more pronounced.

In 1964, outbreaks of ASF were reported in France along the Pyrennes and in the Department of Brittany. Thanks to an early diagnosis and drastic slaughter program in which all infected and exposed animals were eliminated without further efforts to distinguish between ASF and HC, the disease was quickly eradicated. Outbreaks were reported in Italy in 1967 and 1969. Again, drastic slaughter programs were credited with eliminating the disease. The disease has also been reported in Madeira and in 1971 a major incursion of ASF occurred in Cuba. With technical assistance from Russia, France, and Canada, the disease was apparently eradicated in Cuba but only after nearly 400,000 pigs had died or were slaughtered.

At the present time, ASF still persists in Spain and Portugal, and very recently another outbreak was reported in France close to the Spanish border in the vicinity of Bayonne.

II. Signs:

A. Clinical features.--In peracute cases, death sometimes occurs without any apparent signs of disease other than fever and occasionally gross lesions that are hardly apparent. In acute cases, usually there is an abrupt and severe pyrexia which persists for 3 or 4 days. During this period the animals usually continue to eat and appear quite normal. Within 24 to 48 hours before death, temperature of infected swine begins to fall. They stop eating and lie huddled together. The pulse and respiration are accelerated. If forced to rise, they appear weak and poorly coordinated. Cyanotic areas are often present in the skin on the ears and other extremities. Other signs sometimes encountered are mucopurulent nasal and conjunctival discharges, vomiting, and diarrhea. Death usually occurs by the seventh day after the onset of fever.

In the subacute form of ASF, a high fever again marks the onset of the disease. However, the fever may persist for several days or fluctuate irregularly throughout the course of the disease which is usually 3 or 4 weeks. As with the acute form of the disease, there are no features that might clearly distinguish it from HC.

The manifestations of chronic ASF are extremely variable and the disease is usually difficult to recognize. The illness may persist for several months and stunting or emaciation may be the only apparent signs. Pneumonia is the most frequent sign, but occasionally arthritis and large cutaneous ulcerations have been reported. When it has been possible to

observe chronically infected animals throughout the course of the disease, it has been noted that they go through recurring cycles of pyrexia. It is sometimes possible to isolate virus from the blood during the periods of high temperature.

B. Incubation period.--The incubation period following natural or contact exposure is usually 5 to 9 days. In enzootic areas where the disease may be somewhat modified, incubation periods of 8 to 15 days are reported and subacute and chronic infections are more frequent.

III. Pathologic changes:

A. Post-mortem lesions.--In acute ASF, the lesions seen at necropsy are indicative of septicemia characterized by acute infectious splenomegaly and hemorrhages in various organs. There is usually an excess of pericardial, pleural, and peritoneal fluids. Although hemorrhages may occur in nearly every organ, petechiae are frequently observed on the renal cortex beneath the capsule, the mucosa of the urinary bladder, lungs, myocardial, subendocardial, and epicardial surfaces of the heart. The spleen is severely engorged and enlarged; infarction is not observed. Although acute erysipelas may also show infectious splenomegaly and generalized hemorrhages in the organs, petechiae on the renal cortex is more variable in size and irregular in shape. Marked ecchymotic lesions on the gastric mucosa are not seen in acute cases of ASF, while marked reddening of the gastric mucosa is common in acute erysipelas.

Nearly all regional and hilar lymph nodes of organs show swelling and peripheral reddening. The renal and hepatogastric nodes show the most severe hemorrhage and swelling and sometimes resemble blood clots. The mesenteric and pulmonary lymph nodes are less involved. The lungs are

usually edematous. The gall bladder is usually distended with bile and sometimes the wall is edematous. Petechiae are sometimes scattered over the mucosal surfaces and rarely the whole bladder is filled with the clotted mixture of blood and bile. "Paint brush" petechiation is usually present on the gastric and intestinal serosa.

Although the distribution and frequency of the lesions may vary considerably, depending on the strain of virus involved, certain of them occur more often than others and some of the gross lesions when present are considered pathognomonic. In this regard, acute infectious splenomegaly, marked swelling and hemorrhages of lymph nodes, and uniform-sized petechiae no larger than a pinhead on the renal cortex are considered to be most indicative of acute ASF.

In the subacute form of ASF, the incubation period is slightly increased. The disease starts with the onset of high fever. The total course is usually run within 3-4 weeks after infection. As in the acute disease, the reticuloendothelial system is involved and death is possibly due to vascular damage. Hemorrhages are more pronounced in lymph nodes and kidneys. In the latter, the cortex may resemble that of "turkey-egg kidney" and there may be extensive hemorrhage in the pelvices. Moderate edema in the perirenal connective tissue is usually present. Swollen spleen is usually due to hyperplasia of cell elements rather than engorgement. Lobular consolidation is usually observed in the anterior and cardiac lobes of the lungs. The entire lung may be white and does not collapse when the chest cavity is opened. This is due to the presence of diffuse interstitial pneumonia caused by ASF virus infection. Mucosal hemorrhages and bloody contents are often present in the large intestines.

As stated previously, stunting and emaciation are often the only apparent signs in pigs chronically infected with ASFV. Some of these animals may die following one of the frequent recurrences of pyrexia. In such cases, the gross lesions usually resemble those of the subacute disease. Hemorrhages may be prominent, but again any enlargement of the nodes or spleen is due to hyperplasia rather than engorgement.

If the chronically affected animal is killed for necropsy, hyperplasia of lympho-reticular tissue is the most prominent feature noted. The lymph nodes may be considerably enlarged and of very firm consistency. Chronic fibrinous pericarditis and pleuritis are often present. Frequently, firm foci varying in size from a single to several lobules are scattered in the lungs. Often, the lung does not collapse. The nodular lesions may coalesce to form a hard, white mass involving an entire lobe. There are no predilection sites for these foci. The lesions are produced by the infiltration of large numbers of mononuclear cells into the alveolar walls and lumens. Subsequently, the lesions become caseous masses in which calcification may occur.

B. Microlesions.--The virus of ASF acts almost exclusively on cells of the reticuloendothelial system. This is obvious in the histopathology of the acute disease. Severe degenerative changes occur in the lymphoid tissues, including spleen and lymph nodes. Perivascular cuffs of mononuclear cells with frequent karyorrhexis are seen in the brain, and fibrinous necrosis of vascular walls, accompanied by hyperemia and mural thrombiosis are frequently widespread in other organs.

Hyperplasia of lymphoid elements of the reticuloendothelial system are apparent in subacute and chronic ASF. In cases which have died following recurrent fever, degenerative lesions are superimposed on the hyperplastic

changes. The lobular consolidation often seen in the anterior and cardiac lobes of the lungs in the subacute disease show varying degrees of alveolar wall thickening due to mononuclear infiltration; edematous alveoli contain moderate numbers of degenerating mononuclear cells. In the chronic disease where the more advanced necrotic lesions of the lungs are present, the lesions are encapsulated and the adjacent lobules display perivascular and peribronchiolar hyperplasia consisting mostly of plasma cells.

Although the histopathology of ASF is of considerable interest to investigators studying the pathogenesis of the disease in its various forms, it is of little value in differential diagnosis and cannot be relied upon for that purpose.

IV. Diagnosis:

A. In the field.--There are a number of disease conditions in swine which may duplicate some of the signs of ASF, but the main diagnostic problem is in distinguishing it from HC. If epizootiologic events suggest the possibility of an ASF outbreak in an area, any febrile, hemorrhagic syndrome occurring in swine should be regarded as highly suspicious. This attitude should be reinforced by the fact that HC has been reduced to a rarity in this country while ASF has struck as close as Cuba.

Diagnosis of the acute form of the disease as it usually occurs in Africa is not especially difficult and a provisional diagnosis based on the history of the outbreak, the clinical signs, and port-mortem lesions is usually correct. However, the somewhat modified strains of the virus present in areas where ASF has become enzootic in domestic swine usually give rise to a disease that is impossible to differentiate from HC or a number of other swine diseases by clinical signs or pathologic changes. In any case, differentiation requires laboratory confirmation.

B. Laboratory.--A positive diagnosis of ASF requires either detection of the virus or demonstration of the presence of ASF-specific antibody. Several excellent tests are now available, and the evidence required for a positive diagnosis may be detected in any one of several kinds of samples. However, speed sensitivity and accuracy are considerations that govern the selection of samples to be submitted and the test to be conducted.

In acute ASF, the virus may be detected in or isolated from practically every organ, but spleen, liver, lymph nodes, and blood have the highest virus concentrations and are the tissues of choice for diagnosis. For detection of virus, immunofluorescence is the fastest method. Spleen and especially liver are the best tissues for this purpose. In situations where samples may be dispatched to the laboratory within a day or so, pieces of fresh tissue (3 or 4 grams) placed in separate vials and packed in ordinary ice are most satisfactory. About 10 ml of whole blood should also be sent in this manner.

If a longer time is required for transmitting the samples to the laboratory, they should be frozen and shipped on dry ice. In this event, pieces of liver and spleen about a centimeter square should be wrapped separately as cubes in aluminum foil before placing in vials and freezing. Other pieces may be frozen together in a vial. If freezing is necessary, a serum sample should also be sent.

Upon receipt in the laboratory, frozen sections of liver and spleen are cut and stained with fluorescent conjugate prepared from ASF-specific antiserum. Other pieces of tissue are ground together in whole blood to form a suspension that is inoculated on swine leukocyte cultures, and if available normal pigs and pigs immunized against HC are also inoculated.

Serum samples are tested against a preparation of ASF-associated soluble antigens by reverse radial immunodiffusion or immunoelectroosmophoresis to detect the presence of ASF-specific antibodies.

If subacute or chronic ASF are suspected, samples of affected lung tissue, if present, as well as spleen, liver, and lymph nodes should be sent to the laboratory in the same manner as indicated above. A serum sample, however, is absolutely essential, for the detection of antibodies is often the most rapid and certain means of diagnosing the slower forms of ASF.

C. Differential diagnosis.--As indicated previously, pigs dying of acute erysipelas may display post-mortem lesions similar to those of acute ASF, but the petechiae on the renal cortex are quite variable in size and shape while those of ASF are of a uniform size no larger than a pinhead. Also, in contrast to ASF, there is commonly a marked reddening of the gastric mucosa in acute erysipelas. In any case, the laboratory tests readily differentiate the two disease.

V. Prognosis:

Among pigs contracting ASF, mortality is essentially 100%. Although there are occasional survivors, they usually prove to be chronically infected and should be eliminated as potential spreaders of the disease. Slower or apparently milder forms of ASF occur, especially in areas where the disease has become enzootic in domestic swine. Here again, the survivors must be regarded as carriers that should be eliminated if the disease is to be eradicated.

VI. Epizootiology:

A. Geographical distribution.--Outbreaks of ASF have occurred throughout most of Africa south of the Sahara wherever domestic swine have been raised in close proximity to the indigenous wild swine. In parts of Angola and Mozambique the disease is enzootic in domestic swine, and in certain areas of Angola it is said that swine raising is virtually impossible because of ASF. The disease is also enzootic in parts of Spain and Portugal and outbreaks have been reported in Madeira, Italy, and France. The only occurrence of ASF thus far reported in the Western Hemisphere was in Cuba in 1971 and it has apparently been eradicated there.

B. Transmission.--It appears that arthropod vectors or the ingestion of infected tissues may be require for transmission of ASF from the wild to domestic swine in Africa. It is known that at least two species of Argasid ticks not only serve as vectors, but are reservoirs of the virus as well. Once it is established in domestic swine, the disease spreads readily among them by direct contact. The virus present in the excretions and secretions of infected animals is apparently transmitted to other animals by nuzzling or ingestion. The disease does not appear to spread readily by aerosol, for it has been shown experimentally that transmission may not occur between sick and normal pigs kept in separate cages in the same room. However, when the normal pig is allowed to come in contact with the excreta of sick animals, transmission usually occurs. Mechanical spread of the virus can obviously occur. The feeding of raw garbage containing infected meat scraps is an experimentally proven mode of transmission and has been strongly implicated in the spread of ASF from Africa to Portugal and from southern Spain to northern regions of the country.

C. Hosts.--Natural infections with ASFV appear to be confined to porcine species and to certain ticks. In Africa, the virus has been found in wart hogs (Phocochoerus sp.), bush pigs (Potamochoerus sp.), and giant forest hogs (Hylochoerus sp.). These species serve as a reservoir of the virus without displaying signs of illness. Argasid ticks, Ornithodoros moubata porcinus, collected from animal burrows in Africa have been found harboring the virus. In them, the virus replicates and transovarial infection occurs. A similar tick, Ornithodoros erraticus, in Spain has also become infected and has greatly complicated the task of ASF eradication in Spain and Portugal.

The European wild boar (Sus scrofa ferus) is susceptible to ASF and may acquire the infection through contact with infected domestic swine. The response to infection is like that of domestic swine and the lesions are similar.

The virus was apparently isolated in Africa from a hippopotamus, a porcupine, and a hyena. However, these findings have not been substantiated by additional isolations nor have efforts been made to experimentally infect these species.

Among a wide variety of mammals and fowl that have been subjected to virus inoculation, only rabbits and goats have been successfully infected. In both instances, the infections were difficult to produce. Natural infection has not been found in either species.

VII. Control and eradication:

A. Preventive measures.--The first line of defense, of course, should be set against entry of the disease. To this end, there must be a constant awareness and appraisal of the epizootiology of ASF, and importation restrictions must be placed on swine and pork products from

affected areas. All overseas garbage should be destroyed, preferably by incineration, and garbage feeding in general should be restricted to establishments that can assure thorough cooking. Any expertise that is possessed in diagnosing ASF should be freely shared.

It is hoped that the kind of surveillance and reporting of suspicious swine diseases that has been such an important part of the HC eradication program will continue with ASF in mind as well.

B. Sanitation and disinfection.--If an outbreak does occur, the "stamping out" method of eradication must be immediately applied. All sick and exposed animals must be eliminated and disposed of on the affected premises by burning or deep burial. The infected premises should be thoroughly cleaned and disinfected. Manure should be burned or saturated with disinfectant and buried. It has been found that disinfectant solutions containing o-phenylphenol and surface active agents combined are effective in destroying ASFV. In addition, the floors and walls of buildings that have housed swine should, if possible, be thoroughly scalded with live steam.

At least 6 months should elapse before restocking with swine, and during that time, several applications of a pesticide that is capable of destroying ticks is strongly recommended.

All premises surrounding the infected premise must be regarded as being in the affected area and must be kept under close surveillance. The movement of animals from the area must be restricted until it is declared free of the disease.

Every possible effort should be made to prevent the virus from becoming established in an arthropod population. Entomologists especially familiar with the tick species in the area should be consulted.

C. Treatment.--There is no known treatment for ASF.

D. Immunization.--All efforts to produce vaccines with inactivated virus have failed. Several isolates of ASFV have been modified by passage in cell cultures and rabbits and at least two of these have been used as vaccines with disasterous results. They not only failed to protect but quite probably were responsible for the increased incidence of chronic infections now encountered in the areas where they were used.

VIII. Public health aspects:

There have been no reports of ASFV infections in man despite the fact that large quantities of infected pork have been consumed in Africa, Portugal, and Spain and a considerable amount of laboratory work has been done with the virus.

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CONTAGIOUS BOVINE PLEUROPNEUMONIA*

I. Identification of the disease:

A. Definition.--Contagious bovine pleuropneumonia (CBPP), also known as lung sickness of cattle and longsiekte, is a highly infectious disease primarily affecting cattle. The disease may be acute, subacute, or chronic and is characterized by edema of the interlobular and alveolar tissues of the lungs, as well as the sero-fibrinous pleuritis.

B. Etiology.--The causative agent for CBPP is Mycoplasma mycoides var mycoides, also called by the earlier nomenclature, PPLO (pleuropneumonia-like organism). It is extremely pleomorphic and some of its forms are filterable. Mycoplasma are penicillin resistant and grow extracellularly in serum enriched media; colonies have a distinctive "fried-egg" appearance on agar plates.

The disease occurs commonly only in cattle with rare natural cases being observed in buffaloes, yak, bison, reindeer, and antelopes. The causative organism of contagious pleuropneumonias in cattle and goats are similar, but the infections do not spread between the two species.

C. History.--The first records of the existence of CBPP were in Germany in 1693 and in 1743. From here the disease spread in epizootic form over the whole of Europe. The disease was first introduced into the USA in 1843 and eventually resulted in the establishment of the Bureau of Animal Industry in 1884. The disease was completely eradicated from the USA by 1892, from South Africa in 1916, and Australia in 1971. The disease is now primarily confined to Africa and Asia; in Europe it is confined to Spain and Portugal.

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*Prepared by R.J. Yedloutschnig and F.W. Wilder

II. Signs:

A. Clinical features.--In the acute form there is a sudden onset of high temperature (105 F), anorexia, severe depression, and a tendency for animals to stand away from others. Chest pain is evident with the affected animals reluctant to move. Their characteristic stance is with elbows out, back arched, and head extended into the wind. Respirations are shallow and rapid; coughing occurs only when the animal is forced to exercise.

Recovered animals may be clinically normal but they remain carriers. Sequestrae in these carriers may break down upon stress or severe exercise and cause an acute outbreak of the disease. A chronic cough is common. Approximately 50% of affected animals die in the acute stage and about 25% remain as recovered carriers. Death occurs over a course of from several days to 3 weeks.

B. Incubation period.--Under natural conditions the incubation period of the acute form is 3-6 weeks, with an occasional period up to 6 months. The chronic disease may persist for 2 years or longer.

III. Pathologic changes:

A. Post mortem lesions.--Lesions are confined to the thoracic cavity. Pleuritis with heavy deposits of fibrin are seen. One or both lungs may be partially or completely affected with marked consolidation. Interlobular septa are greatly distended with serofibrinous exudate--the classical "marbled" lung. Sequestrae of carriers may be deeply embedded in the lung and no abnormality seen on the surface. Adhesions between pleural surfaces are often seen in such cases.

B. Microlesions.--Lung lobules are frequently separated into distinct compartments by heavily thickened interlobular septa. Some lobules

contain areas of intact alveoli, but in many, consolidation is complete. Intense infiltration with lymphocytes and plasma cells may be noted around blood vessels and bronchi. Leukocytes may be concentrated within the interlobular septa.

IV. Diagnosis:

A. In the field.--Clinical signs are indistinguishable from other diseases which produce a severe pneumonia. Observations of the more or less typical marbled appearance of affected lobules and the excretion of excessive amounts of straw-colored thoracic fluid should lead to suspicion of CBPP.

B. Laboratory.--Serological tests such as complement fixation, precipitin, and agglutination tests are used for laboratory detection of antibodies. Fluorescent antibody techniques give excellent results for detecting the organism in tissues as well as classification by means of colonies on agar.

C. Differential diagnosis.--Any disease producing respiratory problems may be mistaken for CBPP. This includes shipping fever, Pasteurella sp., pneumonia, and many other disease entities.

V. Prognosis:

Death usually occurs in 2 to 3 weeks after the initial signs are observed in the acute disease; mortality varies from 10% to 70%. Animals which do not die may make an apparent recovery; this is frequently nearly half of the affected animals.

VI. Epizootiology:

A. Geographical distribution.--The disease occurs mainly in most parts of Africa and Asia (including Indochina and asiatic USSR). In Africa, the Sudan and Ethiopia have major foci. Cattle in Spain are also affected.

B. Transmission.--The disease is spread through inhalation of aerosols into the respiratory tract. Foci of new outbreaks may result from carrier animals when infective particles are released by respiratory expulsion of material from sequestrae which have broken down.

C. Hosts.--Cattle of all ages are susceptible; most other ruminants are considered resistant. Sheep and goats never contract the natural infection.

VII. Control and eradication:

A. Preventive measures.--Preventing overcrowding and quarantining of apparently sick animals or others which might serve as contact carriers, especially, are important measures. It is especially important to keep recovered animals away from susceptibles because of the extended carrier state.

B. Sanitation and disinfection.--The Mycoplasma are especially susceptible to sunlight and are quickly destroyed in nature. They are not, apparently, spread throughout the carcass. Transmission is largely by aerosol.

C. Treatment.--Sulfonamides and broad spectrum antibiotics have been used in enzootic areas as a means of salvaging meat. However, this should be discouraged because of the production of carrier animals. Slaughter is most economical in small foci of infection.

D. Immunization.--The best vaccines are prepared from live, modified cultures of M. mycoides var mycoides. Several strains are utilized depending on the breed of cattle and the stability of the vaccine. In enzootic areas of CBPP, vaccination will continue to be important.

VIII. Public health aspects:

There is no evidence that man is susceptible.

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DERMOPATHIC BOVINE HERPES VIRUS INFECTION *

I. Identification of disease:

A. Definition.--Dermopathic bovine herpes virus infection (also known as Allerton virus disease, bovine herpes mamillitis, pseudo-lumpy skin disease) is a disease of cattle caused by a herpes virus which may produce a mild to severe mamillitis. It is characterized by a generalized condition of bumps on all parts of the skin not accompanied by other appearance of illness except a mild transient rise in body temperature.

B. Etiology.--The etiologic agent has been identified as a virus belonging to the herpesvirus group.

C. History.--One of the cytopathogenic agents recovered from lumpy skin disease of cattle from Africa was classified as a Group II (Allerton) virus. Bovine mamillitis has been reported in Britain since 1958, but confirmation of a herpesvirus as a causative agent was first announced in 1963. The disease also occurred in Scotland at that time, again in 1964, and with increasing frequency in 1965. During the next five years its frequency decreased. This herpesvirus was next isolated from cattle in the USA in 1970 and from animals in Italy in 1972.

II. Signs:

A. Clinical features.--In Britain and Scotland natural infections have been associated only with mamillitis in dairy cattle. The Allerton virus and isolates from the USA produce a generalized "lumpy skin disease" as well.

B. Incubation period.--In natural infections the incubation period is 5-10 days.

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* Prepared by R.J. Yedloutschnig

III. Pathologic changes:

A. Post mortem lesions.--The major lesions are eruptions of the skin and, where there is mamillitis, inflammation of the dermis of the teats and udder. This may vary or progress from a mild inflammation to an ulcerative condition.

B. Microlesions.--Syncytia formation and necrosis of the epidermis may be found. Many cells and syncytia have central nuclear inclusion bodies.

IV. Diagnosis:

A. In the field.--Demonstration of small nodular eruptions all over the body of bovidae, or inflammation of teats and udder where mamillitis is involved.

B. Laboratory.--Attempts should be made to isolate the herpesvirus and carry out neutralization of the virus in tissue culture. The demonstration of herpesvirus by electron microscopy is helpful. Reproduction of the disease in bovidae confirms the diagnosis.

C. Differential diagnosis.--This disease may be confused with that caused by Dermatophilus congolensis or other fungal or bacterial conditions; urticaria, cowpox, or the true lumpy skin disease (Neethling virus) also cause similar lesions.

V. Prognosis:

Mamillitis cases may be severe, with mastitis; one quarter may even be lost. The lumpy skin condition usually heals with no after effects.

VI. Epizootiology:

A. Geographic distribution.--Allerton virus is present in Africa; another virus causing mamillitis exists in Britain and Scotland. The herpesviruses recently reported from the USA and Italy are all similar.

B. Transmission.--The disease may be transmitted by direct contact and through milking. Biting insects are also considered to be a factor in transmission. Most outbreaks in Britain and Scotland have occurred between June and December.

C. Hosts.--The only natural hosts are apparently the bovidae. Mice and guinea pigs may be infected experimentally.

VII. Control and eradication:

A. Preventive measures.--Quarantine mamillitis cases; these should be milked last and away from the rest of the herd. The population of biting insects should be eliminated or lowered.

B. Sanitation and disinfection.--Equipment and hands should be thoroughly disinfected following use on infected animals.

C. Treatment.--Supportive therapy is recommended for the mamillitis or ensuing mastitis.

E. Immunization.--None.

VIII. Public health aspects:

Authorities are uncertain regarding the possibilities of human infection.

Selected References:

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FOOT-AND-MOUTH DISEASE *

I. Identification of Disease:

A. Definition.--Foot-and-mouth disease (FMD) is a contagious, viral infection primarily of cattle, swine, sheep, and goats, but also of other cloven-footed domestic and wild animals. It is characterized by vesicular lesions and subsequently by erosions of the epithelium of the mouth, nares, muzzle, feet, teats, udder, and rumen pillars.

B. Etiology.--Foot-and-mouth disease virus (FMDV) is classified along with the enteroviruses and rhinoviruses as a member of the picornavirus group. The virus is essentially spherical and is about 23 mμ in diameter.

C. Other vesicular diseases.--The other vesicular disease viruses have the following shapes and approximate diameters: vesicular stomatitis (VS) bullet-shaped, 65 x 175 nm; vesicular exanthema of swine (VES) spherical, 35-40 nm; swine vesicular disease (SVD) spherical, 28-32 nm. All of these viruses contain RNA. FMDV differs from most of the other picornaviruses (except rhinoviruses) by being more labile to pH changes. The organic acids (e.g., acetic acid) and strong bases (e.g., sodium hydroxide) are commonly used as disinfectants for FMDV.

At least 7 immunologically distinct types of FMDV have evolved, and they are identified as types O, A, C, SAT** -1, SAT-2, SAT-3, and Asia-1. Within the 7 types, at least 61 subtypes have been designated by complement fixation (CF) tests conducted by the FMDV World Reference Laboratory at the Pirbright Research Institute, Surrey, England and the Pan American FMD Center, Rio de Janeiro, Brazil.

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There are two immunological types of VSV, New Jersey and Indiana. Three subtypes of Indiana type virus have been identified, only one of which exists in the U.S.

VES virus is more mutagenic for at least 11 different immunologic types of the virus were identified before the disease was eradicated from U.S. swine in 1956.

The swine vesicular disease viruses are currently known only as viral isolates, from Italy, Hong Kong, England, France, Austria, Poland, Belgium, and Japan.

D. History.--The first acknowledged description of FMD was made by the monk physician, Hieronymus Fracastorius, who reported on an epizootic in cattle that occurred near Verona, Italy in 1514. For the next two centuries there was an ever increasing number of epizootics in cattle reported in various parts of Europe, many of which probably were FMD. The disease has long been present in Africa and Asia and most of South America. The United States has had 9 FMD outbreaks between 1870 (Oriskany, N.Y.) and 1929 (Los Angeles, Cal.). The 1914-1916 outbreak was the largest and most costly; FMD invaded 22 states and the District of Columbia, involving about 172,220 animals. The disease last occurred in Canada in 1952 and in Mexico from 1946 to 1954. The Central American countries from Guatemala through Panama have never had FMD, nor has New Zealand. Japan and Australia have been free of FMD for more than 50 years.

II. Signs:

A. Clinical features.--In the respective hosts of FMD, VS, VES, and SVD, the clinical signs and lesions so closely resemble each other that a differential field diagnosis is almost impossible to make. The classical signs of these vesicular diseases are salivation and lameness caused

by the formation of vesicles or blisters in the mouth and on the feet. However, before vesicles form there are usually some obvious signs of illness such as dullness, inappetence, uneasiness, a fall in milk yield in dairy cattle, fever, and sometimes shivering. A few hours later, quivering and smacking of lips, drooling, slight nasal discharge, shaking or kicking out with feet, or lameness may be noticed. After the vesicles have formed, the various signs usually are more pronounced, with salivation and often nasal discharge more copious and lameness more evident. Pregnant cows may abort and young animals may die without showing outward signs of infection. The mortality in mature animals seldom exceeds 5 percent. In young animals, the mortality may be as high as 50 percent.

The diagnostic lesions are epithelial vesicles or blisters. Lesions may be found on the tongue, dental pad, gums, cheeks, hard and soft palate, lips, nostrils, muzzle, snout of pigs, coronary band, corium of dewclaws, interdigital space, teats, udder, rumen pillars, and in the myocardium and skeletal muscles. With the exception of rumen, myocardial and other muscle lesions, VS, VES, and SVD produce lesions in the same anatomical areas as does FMD.

Often FMD lesions are found on all feet, but sometimes only one or two feet are involved. Swine often have lesions on the snout and tongue, but the diagnosis is usually based on the foot lesions. The most common site for oral lesions in sheep is the dental pad. In general, the signs and lesions seen in swine, sheep, and goats are similar to those found in cattle, but they may be less obvious.

B. Incubation period.--When susceptible animals are in contact with infected animals that are in the clinical stage of the disease, transmission

of FMDV readily occurs and recognizable clinical signs of FMD usually may be seen in the exposed animals within 3 to 5 days. Much longer incubation periods also have been reported. The peak period of transmission usually is about the time the vesicles rupture. Pigs fed garbage contaminated with FMDV may show signs of infection in 1 to 3 days. Artifically exposed animals may develop signs as early as 12 hours after inoculation, however, the usual interval is 24 to 48 hours.

III. Pathologic changes:

A. Pathogenesis.--Various studies have shown that the usual primary site of FMD infection and initial virus replication is in the cells of the mucous membrane of the throat.

The usual pathogenesis of FMD in cattle may be summarized as follows: 1) inhalation or ingestion of virus; 2) infection of cells in throat area; 3) replication of virus in throat area and spread to adjacent cells; 4) escape of virus to blood and lymph vessels; 5) infection of lymph nodes and other glands; 6) infection of cells at the sites of predilection for lesion development; 7) virus present in various body fluids; 8) beginning of fever; 9) appearance of oral, nasal, podal, and rumen vesicles; 10) appearance of salivation, nasal discharge, lameness; 11) rupture of vesicles and increased clinical signs; 12) end of fever; 13) end of viremia and start of detectable antibody production; 14) decline of virus titer in various tissues and fluids; 15) healing of lesions and resumption of eating; 16) gradual disappearance of virus in tissues and fluids; 17) healing completed but continued residence of virus in throat area with slow replication resulting in carrier state.

Secondary lesions of FMD are those that appear after there has been an initial lesion elsewhere. For example, if an animal is inoculated with FMDV in the tongue epithelium and this becomes the primary lesion, the other

later appearing oral, nasal, or podal vesicles are called secondary lesions. Teat and udder lesions may be primary as the result of virus contact during milking or by nursing infected young.

B. Postmortem lesions.--Rumen, myocardial, and skeletal muscle lesions occur in FMD infection, but not in VS, VES, or SVD. The rumen lesions (found on the pillars) begin as true vesicles similar to oral lesions, but have a thinner epithelial covering. The FMD myocardial lesions are areas of degeneration and necrosis, not vesicles.

Sometimes, especially in young animals, the so-called "tiger heart", with lesions in the form of stripes or bands, is found. Young animals that have died of FMD infection may have this extreme form of heart involvement.

C. Aftermath of FMD.--As an aftermath of FMD infection, animals may develop chronic secondary infection of their oral, nasal, or podal lesions. Hoof deformation may result in permanent lameness. Also, mammary gland involvement may result in chronic mastitis or a low milk yield. Unthriftiness and failure to regain weight are often seen. Sometimes this is associated with heart damage in FMD infections. Abnormal estrus or breeding problems may last for many months. The panting syndrome has been associated with pituitary gland involvement from FMD infection, resulting in an upset in the body heat regulatory mechanism. Diabetes mellitis also has been found as an aftermath of FMD.

IV. Diagnosis

A. In the field.--The typical vesicles, with blanched epithelial covering and filled with clear, colorless, or straw-colored fluid, are pathognomic of FMD, VS, VES, or SVD, and evidence of their existence is essential in clinical diagnosis of any of these diseases. After rupture of the vesicles, the lesions usually progress through necrotic, ulcerative and fibrinous

exudative stages. Occasionally, there are dry lesions that do not vesiculate. When the lesion is in these other stages or forms, diagnosis becomes difficult because other diseases may produce similar appearing lesions. The clinical signs of vesicular diseases, fever, salivation, nasal discharge, or lameness, are also produced by certain other diseases.

In cattle, oral and nasal lesions of rinderpest, infectious bovine rhinotracheitis, malignant catarrhal fever, viral diarrhea/mucosal disease, bovine herpesvirus infection/bovine mammilitis, lumpy skin disease, bovine papular stomatitis, infectious bovine ulcerative stomatitis, bluetongue, mycotic stomatitis, localized bacterial infections, and photosensitization could be confused with the later stages of FMD or VS. In addition to some of these diseases, foot rot, chorioptic mange, bovine pox, contagious ecthyma, ergot poisoning, and injuries from trauma or chemicals produce foot lesions that may resemble those of FMD or VS. In sheep, the other diseases that may cause diagnostic problems regarding FMD or VS are: sheep pox, bluetongue, contagious ecthyma, lip and leg ulceration and infection with Fusiformis sp. When only swine are involved, FMD, VS, VES, or SVD are clinically indistinguishable from each other and old lesions possibly could be confused with those of swine pox or injuries from trauma or chemicals.

B. Field samples.--Oral, nasal, or podal lesions may be used, but they should be fresh and representative. The following samples may be taken from each of 2 or 3 animals.

1. Vesicular fluid (quantity: all that is obtainable).

2. Vesicular lesion epithelial coverings.

3. Flaps of epithelial tissue still attached to the edges of the lesion (2nd choice)(quantity: from Items 2 and 3: 5.0 gm).* Old necrotic fibrinous material that is difficult to remove is undesirable.
4. Blood with anticoagulant added (quantity: 5.0 ml). Viremia ends about 4 or 5 days after onset of disease.
5. Oesophageal-pharyngeal (OP) fluid obtained with a cup-probang from cattle, sheep, or goats, but not from pigs (quantity: about 10.0 ml, before dilution).
6. Blood for serum samples (quantity: about 10.0 ml of serum).
7. From dead animals, a sample of lymph node, thyroid, adrenal, kidney, or heart may be taken (quantity: about 10.0 gm).

Samples of lesion epithelium, OP fluid, and serum always should be taken. In addition, if there are animals convalescent from the infection, serum samples should be taken from them. With the exception of serum samples, it is important that all other samples are promptly frozen and arrive at the laboratory in that condition.

C. Laboratory.--The field samples, i.e., vesicular fluid or lesion tissues, are prepared as the antigen for complement fixation (CF) tests with guinea pig reference serums for the various vesicular diseases. If the sample contains sufficient virus and it happens to be one of the 4 vesicular diseases, a differential interpretation can be made. In the case of FMD or VS, the virus type will also be known. Additional tests are required for VES and SVD and to determine FMD or VS subtypes. Some of the lesion tissues, OP fluids, blood with anticoagulant and organ tissues are

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*When lesion material is placed in a vial, 5.0 gm occupies a space about equal to 5.0 ml of water.

used for virus isolation. All viral isolates are subjected to CF tests for identification to confirm initial CF test results. Serum samples from convalescent animals may be used to differentiate the vesicular diseases in neutralization, agar gel diffusion or fluorescent antibody tests. These tests also may be used to identify viral isolates.

D. Differential diagnosis.--In the past, differentiation of the vesicular diseases was sometimes possible by animal inoculation, but modern rapid laboratory diagnostic tests has made this unnecessary in the field.

Differential Diagnosis of Vesicular Diseases

Based on Species of Animals Infected.

Diseases	Cattle	Sheep	Swine	Horses	Man
Foot-and-mouth disease	S	S	S	R	S***
Vesicular stomatitis	S*	S	S	S	S**
Vesicular exanthema of swine	R	R	S	E	R
Swine vesicular disease	R	R	S	R	E****

*IM inoculation of VS virus in cattle gives negative results.

S = susceptible, R = resistant, E = experimental transmission only.

**Vesicles rarely occur in man.

***Only a very few cases recorded in world's literature.

****Thus far, laboratory infections only.

V. Prognosis:

Prognosis for recovery in FMDV, VS, VES, or SVD is generally favorable except in very young animals or unusually severe epizootics. From the standpoint of eradication, however, prognosis is an insignificant factor since the first principle of success involves early discovery and prompt disposal of infected and exposed animals.

VI. Epizootiology:

A. Geographical distribution.--Currently (1972), FMD is found, and generally considered enzootic, in Asia, Africa, and most of Europe and South America. North and Central America, Australia, and many of the smaller islands of Oceania are free of FMD. The major livestock producing countries that are also free of FMD include: New Zealand, Japan, the Philippines, Norway, Ireland, and periodically Great Britain. Vesicular stomatitis is confined to the tropical and temperate areas of North, Central, and South America. In recent years, very few outbreaks of VS have occurred in the United States. It is now believed that VES may be an extinct disease in swine that was known to have flourished for only 24 years. The first outbreak of VES was discovered in California in 1932 and the last identified case involving swine occurred in New Jersey in 1956. Swine vesicular disease has been identified in Italy, Hong Kong, England, France, Austria, and Poland.

Interestingly, during the last year workers on the West Coast have isolated several viruses from two species of marine mammals that produce vesicles when inoculated into swine which are indistinguishable from those produced by FMDV, VESV, or SVDV. The chemical and physical properties of the viruses from marine mammals are also indistinguishable from those of VESV. Madin has theorized that marine mammals may be the reservoir for

VESV and that the disease may have originated in swine in California in 1932 as a result of feeding harbor seals to swine.

B. Transmission.--The primary method of transmission of FMDV from infected to susceptible animals is via respiratory aerosols. Aerosol transmission usually occurs with animals in close proximity. However, there is circumstantial evidence that animals may be infected from several yards to many miles downwind from a source of infection. In the British outbreak of FMD in 1967-1968, there is some evidence that contaminated milk aerosols from milk truck vents may have spread the disease to several farms. It has been experimentally demonstrated that when man inhales the respiratory aerosols of FMD-infected animals he may pick up and harbor the virus in his throat area for at least 24 hours. During this time, man may transmit the virus to other people and to animals via his respiratory aerosols. The OP fluids and respiratory aerosols from FMD-infected animals may contain virus before, during, and after appearance of clinical signs and lesions of the disease. Thus, normal appearing animals that have recovered from infection or were vaccinated for FMD and then exposed to the virus may harbor the virus in their throat areas for variable periods of time (about 6 to as long as 24 months in cattle, about 4 to 6 months in sheep and goats, but only during the clinical stage of the disease in pigs). There is circumstantial evidence that such carrier animals, especially cattle, may transmit the disease when introduced into a FMD free herd. However, experimental attempts to demonstrate transmission of FMD from carrier to susceptible animals have been unsuccessful. Experimental transmission of FMD from infected to susceptible animals has been successful only to about 8 days after the onset of the disease in the donor.

In addition to inhalation of virus, animals also may get their initial throat infection of FMDV by ingestion of contaminated forage, grain, animal products, or water or by licking contaminated objects (e.g., a German outbreak was traced to a bicycle). The virus also may gain entrance and establish the initial infection through abrasions in the mucous membranes or skin. It has been demonstrated experimentally that FMD may be transmitted by artificial insemination of infected semen. Meat scraps and bones from infected animals often have been the source of FMD infections in pigs, which then can readily transmit the disease to cattle and other animals. Several United States FMD outbreaks were traced to pigs that had been fed uncooked garbage from foreign ships. Outbreaks or spread of FMD have also been traced to the use of contaminated biological products (usually of foreign origin), such as: vaccinia vaccine, hog cholera vaccine, and pituitary extract. Since FMDV is present in much of the apparently normal skin, the salting, drying, or surface disinfection of hides from infected animals does not preclude virus survival. For short periods, virus may survive in wool. Imported endocrine glands and improperly dried blood are also potential sources of FMDV. Milk from infected animals sometimes contains a considerable amount of FMDV.

Outside the animal body, variable conditions affect virus viability. When exposed to sunlight, especially in a thin layer, the virus is readily destroyed, but in tissue fragments containing the virus or on material, such as hair, feet, and stable equipment, the virus may remain infective for several weeks under the usual stable and farm conditions. In one instance, on a farm in California, the virus persisted for 345 days.

C. Hosts.--Cattle, swine, sheep, goats, water buffalo, bison, deer antelopes, wild swine, reindeer, moose, llama, chamois, alpaca, vicuna, giraffes, and camels are generally considered the natural domestic and wild hosts of FMDV. Experimentally, FMD may be transmitted to mice, rats, guinea

pigs, rabbits, hamsters, embryonating chicken eggs, chickens, and various wild species, including European hedgehogs, chinchillas, muskrats, grissly bears, elephants, armadillos, and peccaries. Horses are resistant. Young dogs and cats may be infected by inoculation of virus, but probably do not contract the infection by natural means. When inoculated, FMDV will replicate without producing clinical signs or lesions of disease in monkeys, turtles, frogs, and snakes. Suckling mice have replaced guinea pigs for many FMDV studies. Guinea pigs are mainly used for production of diagnostic serums and in vaccine potency studies.

Generally, all susceptible animals in an exposed herd develop infection in time, but, under some circumstances, the incidence of disease is considerably less than 100 percent. Young animals are usually more susceptible than adults, unless protected by maternal antibodies arising from previous infection or vaccination.

To reduce viral aerosols in a FMD outbreak, the order of kill of infected and exposed animals should be: pigs, cattle, and sheep. Airborne FMDV may persist in animal rooms for at least 48 hours.

VII. Control and eradication:

A. Preventive measures.--The exclusion of animals of susceptible species and fresh meats therefrom, and restrictions on animal products originating in infected countries, constitute the most effective preventive measures. Included among these measures is the prohibition of garbage containing meats from infected countries aboard ships and aircraft arriving in the United States. Some products considered to be potentially dangerous, such as hides, bones, animal casings, and glands, are permitted entry into the United States but only under prescribed conditions wherein the products are processed at designated official establishments under supervision or comply with other regulations permitting their safe entry.

B. Control and eradication procedures.--

Reporting. If a vesicular disease or any foreign disease of animals is suspected, the owner should be requested to refrain from moving animals or animal products from the premises until the diagnosis is made and the veterinarian should promptly notify the nearest State or Federal veterinarian in his state. A specially trained diagnostician is available and will be sent to assist in the field diagnosis and collection of specimens for submission to the appropriate laboratory.

Special actions applicable to epizootics. In addition to prompt imposition of effective quarantines, immediate establishment of inspection procedures for the purpose of checking all possible contacts and cleaning and disinfection of the affected premises constitute the chief means of combating the disease.

Stamping-out method. Briefly, this technique consists of the following actions:

(1) Promptly slaughtering and disposing of animals infected with or exposed to FMD, thus removing at once the greatest source of active virus and avoiding the possibility of carriers.

(2) Carrying out thorough cleaning and disinfection of the premises and of material possibly contaminated with virus.

(3) Instituting prompt and meticulous tracing of contacts with infected herds and initiating a system of repeated inspections and surveillance in the areas involved.

(4) Imposing rigid judiciously designed quarantines for control of movement of people, livestock, animal products, and feeds.

(5) Indemnifying owners for animals, products, and materials destroyed in the course of eradication procedures.

(6) Testing the contaminated premises 30 days after disinfection by placing test animals, including cattle and hogs, to feed and graze and otherwise come in contact with all parts of the premises and objects which might have been contaminated with FMDV.

Since the 1902 outbreak, the stamping-out or slaughter method of eradication has been the established procedure in the United States where every practical means is used to keep the disease out of the country. If the infection were to enter again despite these protective measures, the same established procedures for eradicating the disease would undoubtedly be applied. General experience has demonstrated the practicality, economy, relative speed, and efficacy of these procedures. The infection has been eliminated through similar efforts in Canada. The British Government has reaffirmed a similar policy, even though the British Isles are almost continuously exposed. It must be realized, however, that elimination of a plague like FMD might present many new and complex problems today. For example, more livestock is being shipped greater distances in more ways and with greater speed than ever before. The control of animal transport is one of the most important functions of the livestock sanitarian. Another of today's problems is the burial and burning of large numbers of animals. Consideration must be given to the effect of such procedures on water and air pollution.

C. Treatment.--There is no known specific cure for the disease and the palliative treatment only alleviates the signs and does not prevent the spread of infection.

D. Immunization.--The use of vaccines in the control of FMD in Europe began about 1938. In Europe and South America, generally trivalent (i.e., with types A, O, and C) vaccines are used. In order to be

effective, vaccines must contain virus of the same type, and frequently of the same subtype, that prevails in the field. Like certain other viruses, FMDV is frequently being changed by mutations, natural passage through various species of animals, and probably by passage through carriers with varying levels of antibody protection. This necessitates frequent checking of at least primary outbreaks and widely scattered outbreaks; often the viral composition of the vaccines must be changed several times during the course of an epizootic.

Vaccinations should be controlled officially in a systematic manner. Under the best conditions, the vaccines for FMD are not infallible. Resistance induced by a good product wanes rapidly after 4 to 6 months; therefore, vaccination must be repeated at intervals. Even with modern laboratory techniques for producing virus for the vaccine, the cost of immunization is substantial. For example, in France, all cattle 5 months or older are vaccinated annually and the total cost for vaccinating one animal is approximately $1.00. In Argentina, the cost for one vaccination is much less; however, many estancias repeat the vaccination 3 times a year.

With ample supplies of safe potent vaccine, substantial control of FMD has been accomplished in several countries, and some have used vaccine as a supplementary, temporary adjunct to eventual eradication. If eradication is the goal, however, vaccination must be terminated eventually in order that the presence of the virus may be revealed by a totally susceptible population. Moreover, strong protective measures must be imposed or the disease may be reintroduced readily from infected areas; inevitably, eradication will be accomplished only through concerted regional or continental efforts.

E. Import Restrictions.--Restrictions on imports of animals and animal products are formulated and administered by the Animal and Plant Health Inspection Service of the U. S. Department of Agriculture, under the authority of Section 306A of the Tariff Act of 1930, and Acts of 1890 and 1903. The regulations are subject to revision from time to time in accordance with the requirements of changing conditions.

VIII. Public health aspects:

While man may harbor FMDV in his throat area for short periods of time, the temporary infection rarely becomes a clinical entity. Thus, the disease is not a public health problem.

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FOWL PLAGUE*

I. Identification of disease:

A. Definition.--Fowl plague (FP), also termed klassische geflugel-pest and peste aviaire, is a highly contagious, acute, fatal disease of chickens, turkeys, pheasants, and waterfowl. Guinea fowls, mynah birds, blackbirds, and sparrows are susceptible.

B. Etiology.--Fowl plague was first reported in Italy in 1878; in 1900, its viral etiology was proved. In 1955, it was shown that FP virus shared a common antigen with influenza A viruses and therefore it was classified as a member of that group of myxoviruses. Fowl plague virus is 80-120 mu in diameter having a single stranded RNA in its core, which is surrounded by a protein coat and an envelope. The viruses of fowl plague, Turkey/England/'63, Chicken/Scotland/'59, Tern/S. Africa/'61 and N virus all are members of the avian influenza A group of viruses. Infections with these viruses (except N virus, which produce 20% mortality in chickens) result in high mortality, up to 100% under natural conditions. However, the relationship among these strains are determined by analysis of the viral envelope antigens. Infectivity is rapidly destroyed by formaldehyde, detergents, halogens, and dilute acids. Fowl plague virus was viable after 15 minutes at 55° C, but was destroyed after 5 minutes at 60° C. At pH 4.0 it is destroyed within 60 minutes. The virus may survive on feathers for at least 18 days and in dried blood and tissues for several weeks. Its infectivity was retained in chilled meat and bone marrow as long as 287 and 303 days respectively.

C. History.--Disease epizootics in chickens, attributed to fowl plague, appeared in Italy just before the beginning of the 19th century

*Prepared by A.H. Dardiri

and shortly it was clinically differentiated from fowl cholera. Since then, it has been detected in Austria, Switzerland, Hungary, Rumania, and Russia. Occasionally, it has spread to France and Holland; it also has been reported in China, India, Indonesia, and Japan. The disease has been diagnosed in Egypt, Israel, and Iran and it may have also occurred in Nigeria. Outbreaks were reported in Argentina in 1922 and in 1924. The disease also appeared in England for the first time in 1924. Two outbreaks were reported in England in 1963 and they were promptly eradicated.

Following illegal introduction of fowl plague virus by a laboratory worker to the United States in 1923, the disease escaped from the laboratory and appeared in the New York City poultry markets in 1924, where it killed about one-half million birds. From there the disease spread to Pennsylvania, New Jersey, Illinois, Michigan, Missouri, West Virginia, and Delaware. The Indiana agent was probably transmitted by contaminated crates, live poultry shipments, and offal from canning plants. The disease was eradicated and controlled by slaughter, decontamination procedures, and rigid quarantine measures. The last outbreak in the United States was reported in Morris County, New Jersey, in 1929, and was eradicated before it spread beyond the limits of the county.

II. Signs:

A. Incubation period.--It is usually 3-7 days, but may be as short as 24 hours in chickens and as long as 6 days in waterfowl.

B. Clinical features.--Fowl plague is suspected in susceptible flocks when several birds are found dead without any signs except blue combs. The disease course is usually rapid in chickens, and the signs are a function of fever and edema. The early signs are general depression,

appearance of ecchymotic spots on the combs, wattle, and unfeathered parts of the legs. The infected birds are reluctant to move and inclined to hide in the dark areas of their pens. Dullness and inappetence are present. The feathers are ruffled with the neck retracted and, when moving, the birds stagger. Edema appears in the combs, wattles, and ear lobes and may also extend to the ventral parts of the head and around the eyes. Edema usually is accompanied by cyanosis of the comb and wattles which appear dark-red or bluish. The eyes are closed and the conjunctiva is congested, swollen, and petechiated. There may be slight puffing of the hock and metatarsal joints resulting from edema. The edematous parts of the body feel warm upon examination. Edema of the facial tissues and the epiglottis may interfere with respiration and affected chickens may open their beaks and gasp for air. Mucus may accumulate in the nasal cavities causing the birds to shake their heads and sneeze forcefully to expel the nasal discharge. Diarrhea may develop and finally the birds lie on their briskets with their head down; coma develops, and death usually occurs in 3-4 days. In certain cases, individual chickens may have convulsions immediately before death.

In turkeys, the disease is characterized by sudden onset of severe congestion of the non-feathered parts of the head, ruffled feathers, dullness in appearance, and slow movements. These signs are more dramatic in the males than in females. Later in the disease, the birds stagger in walking. Profuse white diarrhea is usually noticed. The course of the disease is 2-3 days longer than in the chicken.

The signs in ducks and geese are accompanied by fever, excessive thirst, and prone position. Signs include congestion of the beaks,

conjunctivae and non-feathered parts of the legs, listlessness, inappetence, and whitish-greenish diarrhea. Young birds frequently have convulsions, excitation, and rolling or circling movements. They may walk into fences and there may be occasional torticollis and ataxia. The hock joints are usually edematous and warm and diarrhea is very profuse and common.

III. Pathologic changes:

A. Gross lesions.--Chickens dying from a peracute form of the disease may not have any gross lesions. However, the changes usually found at necropsy are a result of fever and viremia. In the acute form, fully developed lesions may be characteristic when present. The conjunctivas are often congested and petechiated. The nostrils often show accumulation of thick, mucus, which is tinged with blood. Under the skin over the edematous parts of the comb, wattle, ear lobes, and other parts of the head, there may be infiltration of the intradermal and subcutaneous tissues with clear or sanguinous serous fluid. Congestion of the muscles is very noticeable. Hemorrhages that vary in size from that of a pinhead to an ecchymosis are on the surface of the peritoneal fat, peritoneum, serosal surface of the intestines, and heart as well as the dorsal surface of the sternum and pleural surfaces of the chest cavity. Petechial hemorrhages scattered as though they were sprayed over the area with an atomizer on the pleural surface of the sternum, over the epicardium, and in the vicinity of the coronary artery.

Hemorrhages or ecchymoses may be observed on the mucus surface of the proventriculus especially in the portion leading to the gizzard. Petechiae or ecchymoses may be seen in the gizzard surface under the cuticle. The serosal surface of the intestines may have petechia. The intestinal

mucosa may be covered with catarrhal exudate and hemorrhagic changes especially in the area of cecal tonsils. Blood vessels appear engorged, especially in carcasses which are examined soon after death. The lesions found in turkeys, ducks, and geese are similar to those of chickens but often are less severe.

B. Histopathological changes.--The main microscopic changes occurring in field cases were edema, hyperemia, hemorrhages, and foci of prevascular lymphoid cuffing, in myocardium, spleen, lung, brain, and wattles. Parenchymal degeneration and necrosis were present in the spleen, liver, and kidney.

IV. Diagnosis:

A. Field diagnosis.--Fowl plague is suspected when sudden deaths in a susceptible chicken flock are accompanied by edema, cyanosis of the head, hemorrhages in the proventriculus, gizzard, ventral surface of the sternum, coronary area, and fat of the abdominal cavity. However, such disease features are common to other disease conditions such as acute fowl cholera and velogenic Newcastle disease. Therefore, field diagnosis must be confirmed by virologic and serologic methods.

B. Laboratory diagnosis.--The preferred specimens for laboratory diagnosis are two or three carcasses of birds that were killed *in extremis* following the appearance of disease signs. Recent carcasses are also suitable. In the event difficulties are encountered in dispatching whole carcasses, specimens of liver, spleen, kidney, lung, trachea, and bone marrow may be shipped to the laboratory. All specimens should be frozen when they are received at the laboratory. The specimens should be identified properly and clearly and should be accompanied with a complete history

of the flock including any evidence of recent addition of birds to the flock.

The virus is readily isolated from the blood, liver, spleen, kidneys, lungs, and bone marrow. Embryonated chicken eggs, 9-11 days, are inoculated with bacteriologically sterile suspensions usually made from liver or spleen specimens. The amnio-allantoic fluid from dead embryos is then tested for ability to hemagglutinate chicken red blood cells. Inhibition of the hemagglutination reaction by a reference fowl plague antiserum and not by antisera prepared against the antigens of Newcastle disease of influenza A viruses indicates fowl plague infection. In addition, the infected amnio-allantoic fluids are used as an antigen in the virus neutralization test. Inhibition or the decrease of the death rate of embryos inoculated with mixtures of the antigen and fowl plague antiserum confirm the diagnosis of fowl plague virus. Young birds may also be given injections with the tissue extracts of field specimens or the fluids from the infected chicken embryos to prove the infectivity and degree of virulence of the isolated agent. In specimens from areas where fowl plague has not been reported, the isolated agent is identified by both the hemagglutination and the virus neutralization tests as well as by infectivity tests in young chickens. The combined results of the 3 tests will confirm the proper identification of the isolated agent.

C. Differential diagnosis.--Fowl plague may be confused with velogenic Newcastle disease, infections with influenza A viruses, acute fowl cholera and poisoning with phosphorus. Bacteriologic examination will eliminate fowl cholera, streptococcal infection, and pseudotuberculosis. Phosphorus poisoning produces hemorrhagic lesions, but it can be recognized

by the garlic-like odor of the intestinal contents, which also glow in the dark. The agar gel diffusion precipitin test may be used to differentiate fowl plague virus from Newcastle disease virus, but not from the influenza A group of viruses. The hemagglutination inhibition test, if used with limited numbers of test antigens or antisera, may lead to false negative results. The World Health Organization (WHO) has designated reference laboratories and provides them with a set of such antigens and antisera for preliminary identification of avian influenza viruses. The antigens and their antisera included are fowl plague Duck/England/'62, Turkey/Wisconsin/'66, Quail/Italy/1117/'65, Chicken/Scotland/'59, and Newcastle disease viruses.

V. Prognosis:

The prognosis is unfavorable. In natural outbreaks, the infected flocks may die within 7-10 days following the appearance of the signs. The remaining few survivors are immune.

VI. Epizootiology:

A. Geographical distribution.--Fowl plague is known in North Africa, Angola, Ethiopia, parts of Eastern Europe, Taiwan, Korea, and other regions of southeast Asia. In certain of these countries, mixed outbreaks of Newcastle disease and fowl plague occur.

B. Transmission.--Virus is found in all the fluids and tissues of the body and all the excretions and secretions of the sick or dead birds. Virus is very concentrated in the blood. Therefore, the diseased birds or their carcasses are major reservoirs of infection. The virus can remain viable in these carcasses for long periods. Introduction of one or more infected birds into susceptible flocks is followed by an outbreak within

3-7 days from the introduction. The virus may be transmitted directly by contact and indirectly by contaminated equipment as well as personnel who were exposed to infected birds. Infection also results from ingestion of virus with contaminated water or feed. Circumstantial evidence indicates that vectors may transmit the disease and that surviving birds may be temporary carriers and play a part in the spread of the disease.

C. Hosts.--Chickens and turkeys are most susceptible, but ducks, geese, pigeons, canaries, as well as sparrows, blackbirds, guinea fowl, and other wild birds also may contract the disease. Some mammals, including mice, rats, hamsters, guinea pigs, rabbits, ferrets, and monkeys can be infected experimentally.

VII. Control and eradication:

Addition of apparently healthy birds to a susceptible flock of poultry is one of the major factors in transmission of fowl plague. Therefore, recently acquired birds should be isolated and observed for three weeks. This isolation is essential when birds are imported from areas where fowl plague is known to occur. Dead birds should be submitted for diagnosis accompanied by a complete history.

The purchase of birds such as exotic species from enzootic areas is a means of introducing the disease into poultry flocks in this country. Therefore, all imported birds should be quarantined until it is determined that they are free from the disease. If fowl plague is introduced into a flock, depopulation of the sick and exposed birds is the most effective method of eradication. Dead birds should be placed in suitable containers to prevent the spread of the disease by direct and indirect methods. Infected premises should be quarantined and measures enforced to prevent the

movement of exposed birds or spread of infection by man or fomites. The infected premises should be depopulated and decontaminated thoroughly. The diseased birds should be destroyed by methods which are practical and insure inactivation of the virus. Restocking should not be undertaken for at least one month.

C. Immunization.--Various types of killed virus vaccines in general have not been effective. At least 2 vaccinations given a month apart are necessary. Some vaccines containing adjuvants, when inoculated intramuscularly may cause sterile abscesses in the muscle thereby reducing the meat quality. Vaccines containing live virus grown in chicken embryos or human cell culture have given satisfactory protection under experimental conditions. In 1962, a live virus vaccine was developed in Egypt from a virus isolated from a peafowl and was attenuated by passage in pigeon and chicken embryos. Field trials with this vaccine indicated that 80-90% of the vaccinated chickens were protected.

D. Treatment.--There is no known treatment for fowl plague.

VIII. Public health aspects:

There are no reports of infection of laboratory personnel working with fowl plague viruses. However, a virus closely related to fowl plague "Dutch Strain" was isolated from the blood of a person who was suffering from an undiagnosed illness subsequent to his return to this country from the Far and Middle East countries. Therefore, the public health significance of fowl plague infection needs further evaluation.

Fowl plague is a reportable disease in the United States. Signs of the disease should be reported immediately to disease control officials, such as the state veterinarian in the appropriate state or the county agricultural agent.

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LUMPY SKIN DISEASE*

I. Identification of the disease:

A. Definition.--Lumpy skin disease (LSD) (syn. knopvelsiekte, bovine nodular exanthema, pseudourticaria) is an acute infectious disease of cattle cause by a poxvirus. The disease is characterized by fever and eruption of a large number of intradermal nodules of varying size which later undergo necrosis. In severe cases, skin lesion can be accompanied by superficial lymphangitis, lymphadenitis, and edema of the legs.

B. Etiology.--Lumpy skin disease is caused by a poxvirus - prototype is the Neethling strain. The virus has been propagated in cell culture (kidney - lamb, fetal lamb, fetal rabbit, calf; testes - lamb, calf; fetal rabbit skin; baby hamster kidney (BHK/21); vervet monkey kidney (AVK 58); chicken embryo fibroblasts; embryonated eggs). Lamb testes and lamb kidney cell cultures are the most sensitive. On primary isolation CPE develops in 5-14 days or more after infection. Cell culture adapted virus produces intracytoplasmic inclusion bodies 45 hours and CPE 3 days post-inoculation.

The virus is quite stable. It has been isolated from dry necrotic skin lesions in a salted fresh hide and from an air dried hide kept at room temperature for 18 days. Infected cell culture fluid had no significant loss of titer after 5 days at 37°C and was still infective after 6 months at 4°C. Cell culture and nodule suspension contained virus after 5 years storage in dry-ice freezer.

C. History.--Lumpy skin disease was first recognized in Northern Rhodesia in 1929. It was called "pseudo-urticaria" and was believed to be caused by insect bites. Despite rigid quarantine measures, by 1956 it had spread over most of southern and central Africa.

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*Prepared by C.A. Mebus

II. Signs:

A. Clinical features.--Infections vary in severity from inapparent to severe. With apparent infections, the fever is accompanied by the appearances of nodules or lumps in the skin. The number and size of the nodules are a reasonable index of the severity of the disease. In the mild form, there will be only a few small skin nodules. In the severe form, the appearance of numerous intradermal nodules over the body may be accompanied by salivation, oculonasal discharge, and reluctance to move. The nodules appear firm and flat and vary from 0.5 to 4 cm or more in diameter and may coalesce. Nodules occur more frequently on the neck, brisket, back, thighs, legs, perineum, and udder or scrotum. Besides the skin thickening, lesions become visible because the hairs in the lesion tend to stand more erect. In addition to the skin lesions, nodules may appear in the oral and nasal cavities, trachea, muzzle, conjuctiva, on the eyelids, glans penis, and inner surface of the prepuce. Superficial lymph nodes, particularly the prescapular, prefemoral, and parotid nodes are enlarged. As the disease progresses, some skin nodules may resolve, but more typically, the central part of the nodule undergoes necrosis. In some nodules, the necrotic tissue becomes separated from the live tissue by a rim of granulation tissue but remains in place, thus forming a cone-shaped necrotic core (sitfast) or sequestrum within the nodule. If the sequestrum is removed, an ulcer is formed. In other nodules, the necrotic tissue fails to separate, becomes indurated, and persists as a hard intradermal lump for several months. Necrotic skin areas are predisposed to secondary bacterial infections which can cause large ulcers, or extend into structures such as joints, tendonsheaths, or the udder, and cause mastitis. A few animals may develop mild or severe edema of the legs, dewlap, vulva,

udder or scrotum. Pregnant cows may abort and bulls may become temporarily or permanently infertile.

B. Incubation period.--The incubation period after natural exposure is 2 to 4 weeks, but is 1 to 2 weeks after experimental inoculation.

III. Pathological changes:

A. Gross lesions.--In severe cases, the nodules will be visible on the underside of the skin and may extend into the subcutis. The entire thickness of the skin is involved. An incised lesion will appear whitish-grey and may have a necrotic center. Whitish-grey nodules surrounded by an area of hyperemia may also occur in superficial and deep muscles, lungs, renal cortex, and testes. Yellowish-grey lesions surrounded by a zone of hyperemia may occur in the nasal passages, larynx, and trachea. These lesions may undergo necrosis and form ulcers; inhalation of this necrotic debris may cause pnuemonia. Similar lesions may occur in the areas of stratified squamous epithelium in the upper digestive tract. There may also be a generalized lymphadenitis; lymph nodes may be 4 times larger than normal and on section appear pale and edematous.

B. Microlesions.--The histologic appearance of the skin lesions depends upon the stage of the disease when the tissue was collected. In nodules collected soon after eruption, the epithelial cells in the stratum spinosum may be undergoing hydropic degeneration and coalesce to form microvesicles. The epithelial cells adjacent to the microvesicles will be enlarged; the nucleus will appear degenerate; and there may be one or more acidophilic inclusion bodies in the cytoplasm. The inclusions appear to be surrounded by a lightly stained eosinophilic halo. The associated dermal papillae are edematous and more cellular due to a proliferation of reticulo-

endothelial cells. This cellular proliferation extends throughout the dermis and forms thick cuffs around vessels. Scattered necrotic cells in these areas are not uncommon. Occasionally there is invasion and degeneration of vessel walls which results in thrombosis. Necrotic areas of skin probably result from this disturbed circulation.

IV. Diagnosis:

A. In the field.--A febrile disease associated with the development of skin nodules which undergo necrosis should arouse suspicion of lumpy skin disease. Confirmation of a diagnosis requires laboratory assistance.

B. Laboratory.--Laboratory assistance is needed to differentiate the various conditions that cause cutaneous nodules. Pieces from biopsies of 2 or more early skin lesions and enlarged lymph nodes should be submitted frozen for virus isolation and in 10% buffered formalin for histologic examination. Serologic confirmation can be obtained using acute and convalescent serums.

C. Differential diagnosis.--A number of conditions cause nodules or lumps in the skin and must be considered in a different diagnosis of lumpy skin disease. Dermopathic bovine herpes virus infections (Allerton virus, bovine herpes mammillitis) may closely resemble lumpy skin disease and occurs in epizootic form; in the past, this disease has been confused with lumpy skin disease. Other conditions to be considered are urticarial swellings, streptothricosis, insect bites and parasites.

V. Prognosis:

The majority of animals recover spontaneously. Mortality is usually less than 2%. However, on some farms in Africa, 75% of the cattle died; secondary bacterial infection might have been responsible for this high death rate.

VI. Epizootiology:

A. Geographic distribution.--To date, lumpy skin disease is confined to Africa.

B. Transmission.--The mode of transmission of lumpy skin disease is not known. However, there is ample circumstantial evidence that biting insects play an important role in transmission. In Africa, spread of the disease could not be prevented by quarantine; the disease spread to herds that had no known contact with infected animals. The disease is more prevalent in wet summer months and in areas where there are many mosquitoes. The virus has been isolated from flies caught on infected cattle. Spread by direct contact and fomites has been reported, but this has not been observed to occur under experimental conditions.

C. Hosts.--Bovidae are the only known natural host.

D. Economic importance.--The economic importance of lumpy skin disease results from losses due to secondary infections, infertility, reduced milk production, cachexia and damaged hides.

VII. Control and prevention:

A. Preventive Measures.--Even though measures have not been successful in controlling the disease in Africa, slaughter of infected animals, restriction of stock movement, and spraying with insecticides have been of value in confining the disease.

B. Sanitation and disinfection.--Since virus has been demonstrated in skin scrapings and saliva, animal facilities should be cleaned and disinfected after removal of infected animals.

C. Treatment.--Treatment should be directed against secondary infections.

D. Immunization.--Information about duration of immunity after natural infection or vaccination is confusing. Reports of field observations suggest that the duration of immunity is short (less than 11 months). However, it is believed that in these cases skin lesions were due to different diseases. Serum neutralizing antibodies persist for at least 5 years.

In countries where the disease is enzootic, vaccination is the only method of control. Cattle inoculated with sheep pox virus are protected against lumpy skin. However, this type of vaccine is not practical in countries free of sheep pox. Also available is a cell culture propagated attenuated Neethling strain of lumpy skin disease virus. About 50% of the vaccinated cattle develop a local swelling. The disease does not generalize and the nodule disappears within 4-6 weeks without complications.

VIII. Public health aspects:

There is no report of lumpy skin disease virus infecting man.

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MALIGNANT CATARRHAL FEVER*

I. Identification of the disease:

A. Definition.--Malignant catarrhal fever (MCF) is also known by other terms in various parts of the world. Snotsiekte is an old name still used in Africa. The Animal Health Yearbook of the Food and Agricultural Organization (FAO) of the United Nations lists four official designations of this disease:

Bovine Malignant Catarrh (Code Bb)
Coryza gangreneux des bovins
Fiebre catarrhal maligna
Coryza gangraenosa bovum

The disease affects cattle, buffaloes, American bison and deer of several species. Acute MCF is a generalized disease characterized by high fever, profuse nasal discharge, severe hyperemia, diffuse necrosis of oral and nasal mucosae, leukopenia, ophthalmia, corneal opacity and enlargement of lymph nodes. The clinical syndromes may be divided into four forms, namely the peracute, intestinal, head and eye, and mild. The naturally acquired disease is usually of the head and eye form and has low morbidity and mortality rates.

B. Etiology.--The etiologic agent (or agents) causing many cases and outbreaks of MCF in various parts of the world have not yet been identified. But several strains of a herpesvirus which causes the classic disease have been isolated from species of wildebeest and hartebeest (Plowright, et al, 1960, Mettam, 1923). These African strains are the basis of this discussion. However, the disease induced by African MCFV cannot be distinguished from those caused by etiologic agents as yet unknown.

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*Prepared by M. Kalunda and D.H. Ferris

Size and Shape.--Seen in electron microscopy, the African MCFV has a rounded profile of about 90 millimicrons (mu) in diameter. Each particle consists of an internal dense body or nucleoid separated by a clear zone from a single dense peripheral ring. The nucleoid is about 40 mu in diameter. It may have a pale center, giving it an annular profile on section, although at times it is uniformly dense. It is filterable through a membrane of 430 mu pore diameter. Neither 5-iodo-2'-deoxyuridine (IUDR) nor 5-fluoro-2'-deoxyuridine (FUDR) has any toxic effect on thyroid cells but in the presence of IUDR the cytopathogenicity (CPE) of the virus may be delayed, suggesting that it is a DNA-containing virus. Staining with acridine orange and use of Fuelgen staining also support the concept that it is a DNA virus. It is sensitive to both ether and chloroform.

Classification.--The Western Hemisphere Committee on Animal Virus Characterization places the reference virus strain, WC11, Malignant Catarrhal fever (Wildebeest-Africa) in the family Herpesviridae (DNA). See Am. J. Vet. Res., Vol. 36 (1975), pages 861 to 872.

Bovine thyroid cultures are so far the best medium for the isolation and propagation of the virulent strain. It produces focal CPE marked by syncytial formation and vacuolation. Intranuclear inclusions may also be formed. The CPE becomes extensive on serial passages. The virulent virus is distinguishable from other herpesviruses of cattle by its pathogenicity and the absence of cell-free infectivity in cattle tissues or in early cell culture passages.

Virulent MCFV is modified by cell culture passages to become cell-free and thus stable under various conditions, including freezing and lyophilization. The modified or attenuated agent has a broader spectrum of infectivity in cell cultures. It produces CPE in thyroid cultures characterized by rounding of individual cells and detachment of affected cells from culture vessels.

Cattle inoculated with cell-free strains may show the mild form of the disease, if any, rather than the acute forms induced by the virulent virus.

C. History.--Malignant catarrhal fever is a world-wide disease and usually occurs sporadically. However, epizootics of MCF that have involved large numbers of cattle and deer have been reported from widely separated parts of the world. A recent epizootic of MCF in the USA occurred in cattle in Colorado during the winter of 1971-1972. In one group of 231 cattle, 87 (37%) died within 68 days.

The sheep associated disease was first reported in Europe in 1798; Switzerland, 1832; USA, 1920; and Canada, 1924. The wildebeest associated MCF was known to hunters in the southern part of Africa during the early half of the 19th century. Their observation that this disease was acquired by cattle grazed with black wildebeest was reported in 1850.

II. Signs:

A. Clinical features.--The clinical picture of MCF is arbitrarily divided into four forms, the peracute, intestinal, head and eye and mild forms. But, subdivision of the clinical manifestations into these forms has little practical use since there is considerable overlap in the syndrome observed. It does serve to emphasize, however, that the clinical syndrome can be quite variable and the diagnosis elusive.

(1) Peracute form: Severe inflamation of the oral and nasal mucosa and hemorrhagic gastro-enteritis are observed. The course of this form is 1-3 days.

(2) Intestinal form: This form is characterized by pyrexia, diarrhea, severe hyperemia of the oral and nasal mucosa. Nasal and ocular discharge as well as enlargement of lymph nodes are common features. The course of this form is 4-9 days.

(3) Head and eye form: This is the typical clinical syndrome of MCF. The first evidence of infection is pyrexia, often heralded 2-7 days earlier by nasal and ocular discharges. Bilateral nasal discharge begins as serous and soon becomes mucoid, mucopurulent and later purulent. Encrustation is common in late stages and causes partial or complete blockage of nostrils resulting in dyspnoea. At this stage the sick animal breathes through its mouth and usually shows drooling of saliva.

The oral mucosa exhibits intense hyperemia and diffuse superficial necrosis. Because the basal layer of the epithelium is rarely involved, the necrotic lesions are designated as erosions rather than ulcers. In the live animals, these lesions have a pink or red color due to exposure of the underlying capillary bed. They are found on the lips, gums, hard and soft palate and the mucosa of cheeks. The sharp-pointed buccal papillae are often involved and the tips slough leaving characteristic reddened blunted papillae. Petechiae of oral tissues are occasionally present. These changes cause severe pain and the animal objects to the examination of its mouth.

Changes in the eye include lacrimation that becomes purulent in late stages. Ophthalmia, prominent scleral veins and swollen eyelids are common features. Corneal opacity starts at the periphery and progresses centripetally resulting in either partial or complete blindness. Corneal opacity is usually bilateral but occasionally one eye is affected more severly than the other. Photophobia is usually associated with corneal opacity. An animal exhibiting this sign closes its eye most of the time and points its head away from the source of light.

Pyrexia is a common sign of the disease and is often biphasic. The temperature is usually high, 104-107 C, and remains high until shortly before

death at which time, it is subnormal.

Increased thirst starts in early stages of the disease and continues until shortly before death. Anorexia is observed in the late stages of MCF. Constipitation is a common feature of the head and eye form but terminal diarrhea is occasionally observed.

Nervous signs are rare although shivering, incordinated movements and terminal nystagmus may be observed. Skin lesions are rare. The course of this form is usually 7-14 days, but may be longer (Table 1).

(4) Mild forms: These are syndromes caused by experimental infection of cattle using modified viruses. They are often followed by recovery.

B. Incubation Period.--The incubation period of MCF under natural conditions is not known. The experimental incubation period varies from 9 days to 2 months, depending on the virulence of the virus used and other factors (Table 1).

III. Pathological Changes:

A. Postmortem lesions.--The lesions vary according to the form and the course of the disease. Thus, animals that die of the peracute disease usually show no diagnostic changes. In this case the diagnosis must rest on the detection of the characteristic histopathological changes and positive results of transmission experiments.

In cases of the instestinal or head and eye form, the carcass may be normal, dehydrated or emaciated, depending on the course of the disease. The muzzle is often heavily encrusted and if wiped reveals an irregular raw surface.

The respiratory system may show minor or severe lesions. There may only be a slight serous or a copious mucopurulent discharge. When the course is short, the nasal mucosa shows congestion and slight to moderate serous

TABLE 1

SOME PUBLISHED TIME RANGES, INCUBATION PERIOD AND COURSE OF DISEASE IN EXPERIMENTAL MALIGNANT CATARRHAL FEVER

Experimenter(s)	No. Animals	Incubation Period*	Course Range*
Kalunda, Dardiri (1975)	16 Calves	14 to 26	4 to 52
	31 Steers	16 to 130	5 to 42
Daubney, Hudson (1936)	100+	(No Data)	4 to 60
Piercy (1952)	100+	14 to 37	(No Data)
Mettam (1923)	(No Data)	10 to 34	(No Data)
Merchant, Barner (1964 Review)	(No Data)	16 to 300+	4 to 14
Plowright, et al. (1965)	(No Data)	63 to 77**	(No Data)
Rweyemamu, et al. (1976)	1	150	10

*Period in days.

**Strain from blue wildebeest; cell-free virus which produced fatal disease when cattle were held from 9 to 11 weeks after inoculation.

***This animal was vaccinated with an attenuated strain, challenged and thought to have been protected. Five months later the animal had a 4 month long persistent viremia and died with classical signs of MCF.

exudate. Later, there is a profuse purulent discharge. The mucosa is then intensely congested and edematous. Erosions may be common. Occasionally croupous pseudomembranes form and if these are removed, raw surfaces remain. Turbinates are severely inflamed and often carry pseudomembraneous exudates. The pharyngeal and laryngeal mucosae are hyperemic, swollen and later develop multiple erosions or ulcers. These lesions are often covered in part by a greyish-yellow exudate. The tracheo-bronchial mucosa is congested and usually petechiated; ulcerations may occur. The lungs are normal in peracute cases but may be emphysematous in others. Bronchopneumonia may complicate chronic cases.

The alimentary mucosa may show no significant lesions in the peracute disease. Hyperemia and diffuse superficial necrosis is a common feature in other forms of the disease. The erosive lesions often involve the tips of buccal papillae, gingivae, both divisions of the palate and the cheeks. The tongue is often normal. The esophagus may show congestion, erosions and pseudomembranes. The rumen, reticulum and omasum may have areas of congestion but do not carry lesions. The abomasal mucosa is usually hyperemic, edematous and may carry petechiae. Hemorrhagic ulcerations are also common, especially in the pyloric region. The wall of the small intestine is firm and thickened by edema. The serosa may carry petechiae. The first half of intestinal mucosa may show severe congestion with blood-tinged contents. These changes decrease gradually towards the large intestine. Peyer's patches are usually normal but may show superficial necrosis. The large intestines often show minimal changes, mainly lines of congestion along the longitudinal mucosal rugae. Contents of the large intestine are scant and may be dry and pasty or stained with blood.

Characteristic lesions may appear on the kidneys. They are not always seen but when present are typical. They are usually small (2-4 mm) foci of nonsuppurative intestinal nephritis. These foci form slight rounded projections from the capsular surfaces. They are whitish and represent infiltration of mononuclear cells. The urinary bladder is often normal or its mucosa may be congested.

The liver is slightly enlarged and may have miliary white foci. The gall bladder is distended but otherwise normal. The spleen is often enlarged and the Malpighian corpuscles are prominent. The heart may have petechiae on the coronary groove; the endocardium may show white patches.

All lymph nodes are usually affected but the abdominal ones are less consistently involved than those of the periphery, particulary those of the head and neck. Affected lymph glands are many times the normal size, usually 2-5 times (but occasionally up to 10 times) and are usually hemorrhagic. Some (including hemolymph nodes) are usually too small to recognize, but under the influence of the disease, becomes quite obvious.

B. Microlesions.--Confirmation of MCF and differentiation from similar diseases are based on the histopathological changes. These changes are pathognomonic and are found in lymphoid tissues and in the adventitia and walls of small blood vessels in any organ. They are the same in rabbits to which the disease can be transmitted.

The vascular lesions take the form of a fibrinoid necrotizing vasculitis and cellular accumulation in the adventitia. These lesions are focal and are seen in all cases of MCF regardless of the form of disease.

IV. Diagnosis: See Appendix A, in addition.

A. In the field.--The history of the disease indicating close contact between the infected animal and calving wildebeests in Africa or lambing ewes elsewhere, aids a tentative diagnosis. The long incubation period of this disease, however, often shadows the association between the natural and alien hosts of MCF. Typical clinical features help in forming a presumptive diagnosis. These include high temperature, profuse nasal discharge, severe congestion and diffuse necrosis of oral and nasal mucosae, ophthalmia, corneal opacity and gross enlargement of peropheral lymph nodes. One or more animals in a herd are usually affected in the normal pattern of MCF.

B. Laboratory diagnosis.--See Appendix A, in addition. Specimens required for laboratory examinations in the study of MCF are:

(1) Blood for virus isolation and cell counts. Blood (about 500 ml) should be collected in EDTA (1 mg of EDTA per 1 ml of blood) or heparin.

(2) Tissues for virus isolation. Spleen, lymph nodes, adrenals, tonsils, and thyroids are suitable for virus isolation.

(3) Nasal swabs for virus isolation.

Blood and tissues for virus isolation should be refrigerated but not frozen and should be sent to the laboratory as soon as possible. Buffy coat or cell suspensions from these tissues are inoculated onto established thyroid cultures. These are checked for the typical CPE that may be formed 4-20 days after inoculation. Primary cultures of thyroid, adrenal, kidney and tests cells from infected animals show the typical CPE. But no CPE is observed in primary cultures of buffy coat or lymph nodes from the same animal, although these tissues have the highest titers.

(4) Tissues for histopathological studies. Thin slices of kidney, spleen, liver, adrenals and lymph nodes are fixed in 10% formalin in physiological saline or PBS.

(5) Serum for serological tests (or blood collected for serum). Paired serums are required, the first collected at the onset of the disease and the second during convalescence, if possible. The course of the disease varies a great deal. Blood taken when the animal is near death, or even shortly after death, may constitute the second sample.

C. Differential diagnosis.--The clinical syndrome of MCF resembles that of other diseases especially those that cause necrosis, ulcerations and erosions of the oral mucosa of cattle. Differential diagnosis should therefore include bluetongue, bovine viral diarrhea-mucosal disease (BVD-MD), rinderpest, vesicular diseases and ingestion of caustic substances.

(1) Bluetongue: The clinical reactions of MCF resemble bluetongue especially in the diffuse necrosis of oral mucosa and crusting of the muzzle. Lameness common in bluetongue is absent in MCF and ophthalmia and corneal opacity often associated with MCF are rare in bluetongue.

Virological, serological and histopathological examinations are essential for differential diagnosis of these diseases.

(2) BVD-MD: The classic clinical syndrome of BVD-MD occurs sporadically and is characterized by fever, leukopenia, diarrhea, lacrimation, nasal discharge and erosions of the oral mucosa.

Oral lesions in this disease, unlike those of MCF, are discrete, rounded or linear depressions. Severe hyperemia and ophthalmia, common in MCF are not observed in BVD-MD. Diarrhea is also rare in MCF.

Final differential diagnosis requires virological, serological and histopathological tests.*

*Note: Relationships between African MCF and those of unknown etiology are under intensive research at PIADC. Some relationships will be discussed in Course sessions.

(3) Rinderpest: Rinderpest, enzootic in Africa and parts of Asia is exotic in this country. The clinical syndrome of rinderpest is similar to that of BVD-MD. The introduction of rinderpest virus into the highly susceptible bovine population of USA would result in high morbidity and mortality rates, rapid transmission between animals and herds and a disease generally more drastic than that of MCF. Mild strains of rinderpest virus could easily be misdiagnosed as the mild form of MCF.

(4) Vesicular disease: i.e., FMD and vesicular stomatitis are excluded on the ground that these diseases elicit vesicles on the oral mucosae, teats, and coronary bands of cattle. These vesicles rupture quickly leaving flaps of epithelium.

V. Prognosis:

Prognosis is poor for MCF. The case fatality rate of MCF is 90-100% in Africa while that of a major Colorado epizootic of MCF was 30-40%.

VI. Epizootiology:

A. Geographical Distribution.--MCF has been reported in many countries all over the world.

B. Transmission.--This disease is transmitted from the natural reservoirs to cattle, the alien host. Blue wildebeest (Connochaetes taurinus) and the black wildebeest (Connochaetes gnu) are two of the natural hosts of the MCF virus in Africa. They are inapparently infected. Sheep may be natural carriers of a similar virus in other continents. In the natural disease transmission may occur when cattle graze with calving wildebeest or are housed with lambing ewes. Close contact between the donor and the recipient is presumed to be essential factor in transmission. Transmission of MCF between sick and other susceptible cattle has never been reported.

The mode of transmission and the association of calving wildebeest (or, possibly, lambing ewes) with the infection of this disease have not yet been defined. The latest studies conducted independently on cattle at this laboratory and on wildebeest in Africa have shown that in both cases nasal discharges of infected animals carry the MCF virus. This finding partly explains how cattle could acquire the infection by grazing with wildebeest and indicates that under certain conditions the disease could be transmitted among cattle by close contact.

C. Host Range.--Infection with the virus and clinical signs of MCF have been reported in the following species: cattle, buffaloes, Pere David's deer, American deer, bison and rabbits. Preliminary studies at this laboratory have shown for the first time that sheep can be infected with the wildebeest strain of MCF virus. This has not yet been demonstrated by blood passage with other forms.

VII. Control and Eradication:

A. Preventive measures.--Our present knowledge of MCF dictates that the incidence of this disease can be reduced by separating cattle and the natural hosts during the lambing or calving seasons.

B. Natural Immunity.--Cattle or rabbits recovered from this disease develop solid immunity against all strains of MCF virus.

C. Induced Immunity.--All strains of the wildebeest MCF virus are homologous by the virus neutralization (VN) test. No effective vaccine is available for this disease.

VIII. Public Health Aspects:

The disease is not transmissible to man.

APPENDIX A

TYPICAL SEQUENTIAL PROTOCOL FOR DIAGNOSIS OF
AFRICAN MALIGNANT CATARRHAL FEVER (PIADC)*

Day of Receipt (DOR) Day Post Receipt (DPR)	Specimens Received	Purpose of Specimens	Test No.	LABORATORY TEST OR ACTION	FURTHER ACTION
DOR	1. Refrigerated: Blood in EDTA or Neparin	Virus Isolation	1.	Centrifuge blood at 500 x g 45 minutes; remove BC, wash, re-suspend in medium; inoculate 2 ml on BTh cells.	
DOR	2. Frozen Sera	Serology	2.	CF test for RP, MCF, bluetongue BVD-MD, vesicular disease.	Report if positive for any.
DOR	3. Probang Fluid, Oral or Skin Les. (Frozen)	Serology	3.	CF test for vesicular diseases, using specimens as antigen.	Report if positive for any vesicular disease.
DOR	4. Formalinized: Kidney Spleen Liver Adrenal Lymph Node Brain	Histo-pathology	4.	Prepare microscopic slides and stain with H & E. Typical MCF lesions from positive animals will be found in tissues, regard-less of agent. (Standard method in most laboratories)	Examine under microscope. A positive re-port does not indicate eti-ology.

*Prepared by D.H. Ferris for READEO Meeting.

APPENDIX A-2

	Specimens	Purpose	Test No.	LABORATORY TEST OR ACTION	FURTHER ACTION
DOR	5. Refrigerated living: Thyroid Adrenal Kidney Testis	Demonstrate Typical CPE in Primary Cultures	5.	Prepare primary cell cultures from suspect tissue and seed in Leighton tubes with coverslips.	Read cultures daily for CPE. Pass suspect and unchanged cultures up to 10 times. (Read until controls deteriorate.)
DOR	6. Refrigerated Spleen Lymph Nodes Adrenals Thyroid Kidney Testis Tonsil Nasal Swabs	Virus Isolation	6.	Prepare 10% triturate of each tissue in medium; inoculate 10^0, 10^{-1}, 10^{-2} dilutions on BTh cells. Incubate 1 hour at 37 C, wash off, feed cells.	Read cultures daily for CPE. Pass as in 5 above. NOTE: triturate by mincing for cell-associated African MCFV by Ten Broeck grinders for possible cell virus.
DOR	7. Frozen Sera (Paired)	Virus Neutralization	7.	Using BTh cells in Leighton tubes prepared in lab, perform virus neutralization test per protocols in lab directions.	Read cultures daily, report results.
DOR	8. Refrigerated: Blood Lymph Nodes Spleen	Demonstration of Disease	8.	Inoculate steers, IV and in prescap. LN with 10% triturate of tissues and whole blood (EDTA).	Hold for at least 70 days (300 if possible). Continue to pass to steers or calves by blood from animal showing signs. Continue efforts to isolate agent.
DOR	9. Frozen Sera	FA Blocking Test	9.	Using deep frozen attenuated MCFV on coverslips, perform FA blocking test.	

*This is probably the most effective measure available. From 50 to 500 ml of blood should be used. African MCF can be readily passed by blood indefinitely; MCF's, unknown etiology have been passed several times, but are usually lost eventually. The more passages made, the better the chance of isolating an agent.

APPENDIX A-3

	Specimens	Purpose	Test No.	LABORATORY TEST OR ACTION	FURTHER ACTION
1 DPR	BC cultures on BTh cells	Virus Isolation	1.	Wash the BTh monolayer with PBS to remove BC cells and feed.	
	CF Test for RP, MCF, Bluetongue, BVD-MD, vesicular diseases (overnight version)	Serologic Test	2.	Read and record results of CF test.	Report any positives.
	Primary cell cultures of original tis.	Observe CPE Virus Isolation	5.	Examine cell cultures; too early for CPE.	From 1-14 DPR, read daily; feed as required. Report if & when positive. Pass
	BTh cells inoculated with suspect matter	CPE, Virus Isolation	6.	Examine culture, as above.	As above.
	Leighton tube BTh, virus neutralization test	Serologic Test	7.	Read CPE in neutralization test; calculate neutralization index by Karber method.	Report if positive; but may indicate recovered animal (few!)
2 DPR - 70 DPR (or longer)	Inoculated cattle, rabbits	Demonstration of Disease	8.	Examine and temperature animals daily. Incubation period of calves, steers was 14-16 days, with a mean of 22 days. The incubation period of rabbits was 17 $\pm$ 5 days.	Hold and observe as long as possible. At pyrexia or other signs, pass blood to other animals. (Data refers to Kalunda's work at PIADC; incubation period can be longer)

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NEWCASTLE DISEASE*

I. Identification of the disease:

A. Definition.--Newcastle disease (ND) is also known as pseudoplague of fowl, pseudofowl pest velogenic viscerotropic Newcastle disease (VVND), and Ranskeldisease. It is a highly contagious and destructive viral disease which attacks chiefly chickens and turkeys. Other poultry and feral birds as well as man may contract the infection.

B. Etiology.--Newcastle disease is caused by a ribonucleic acid (RNA) paramyxovirus of a size estimated to be 80 to 180 nm. The virus has the ability to agglutinate and lyse chicken erythrocytes (among those of other species), a characteristic which may be inhibited by ND specific antiserum. The virus may be inactivated in 1 minute at 100 C and in 30 minutes at 60 C. At 37 C NDV activity recedes in a matter of days. The virus is destroyed by ultraviolet light as well as several chemical disinfectants, among these formalin (0.5%) inactivates the virus in approximately 18 hours.

Although the virus is adversely affected by warm temperature and solar radiation, it may be protected from these influences by its proteinacious coating. Therefore cleaning and sanitization of contaminated surfaces and objects before application of disinfectants facilitates their effectiveness.

Newcastle disease strains are further classified on the basis of their virulence into velogenic, mesogenic, and lentogenic strains. Velogenic strains are exotic to poultry flocks in the USA, but make incursions into the country occasionally.

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*With particular reference to exotic forms. Prepared by A. H. Dardiri

C. History.--The disease was first described by Kraneveld in Java (1926) followed by the report of Doyle in 1927 of the malady in a flock of chickens in Newcastle, England. By 1940 ND was reported from the Philippines, Asia, Australia, and Africa; it was later reported from the continent of Europe. The initial form of the disease was characterized by extensive hemorrhage of the digestive tract. Other characteristics caused confusion with fowl plague at times. In 1944 ND was identified in California and then on the East Coast of the USA. Through the years there have been outbreaks in many poultry-raising areas of the Western Hemisphere. Usually the disease encountered was mild and accompanied by respiratory and neurological disorders which lead to the term "pneumoencephalitis" being applied.

On several occasions a more severe form of the disease appeared in the USA, mainly through the importation of exotic birds. The most recent incident was the 1972 outbreak in California which required a costly program of vaccination and "stamping out" procedures to effect eradication. This form of the disease was referred to as velogenic viscerotropic Newcastle disease (VVND). Approximately 40 million chickens were destroyed and the approximate cost of eradication was 58 million dollars.

II. Signs:

A. Clinical features.--An outbreak of VVND may be so acute and severe as to kill nearly all of a flock within 3 or 4 days. In the peracute form the birds die suddenly without noticeable signs. More often in the acute form, the birds first appear listless, their respiratory rate increases, a pyrexia appears, weakness becomes apparent, followed by prostration and death in 5 to 7 days. The sick birds may display watery, greenish diarrhea which is sometimes blood stained and profuse. As a result of the fever and

diarrhea, the birds appear dehydrated. A rise of 4-6 C may occur early and then subside to below normal levels before death. Clonic spasms and torticollis may appear in birds that survive. Mortality is usually 90% to 100%.

Frequently some chickens show cyanosis of the comb and wattles and edema of these organs as well as the area extending from between the wattles and the upper part of the neck. There is considerable variability in the severity of signs. This variation is influenced by the species, age, and natural resistance of the birds as well as the potency of the viral strain.

B. Incubation period.--The incubation period is usually 2 to 6 days but may be as short as 2 or 3 days.

III. Pathologic changes:

A. Post-mortem lesions.--The mouth usually contains mucus discharges which may be tinged with blood. Dark cyanotic combs may be found on birds at death. Facial and neck edema may be severe, especially in young birds. Straw-colored exudate may be excreted from the eyes and nasal openings. A diphtheritic pharyngitis may be present. Occasionally edema is present in the subcutaneous tissue of the face, entrance of the thorax, or at the end of the keel. The tracheal lesions are usually hemorrhagic without free blood in the lumen of the trachea. Occasionally the lining of the proventriculus is hemorrhagic as well as the serosal surface of the organ. Upon removal of the lining, the surface of the gizzard may be found hemorrhagic. Numerous small hemorrhages are frequently found in the intestine.

The most constant necropsy finding in velogenic Newcastle disease is occurrence of hemorrhagic lymphoid foci in the intestines. These occur

in the duodenal end and also prominently in the cecal tonsils. Lymphoid plaques or patches may be seen protruding on the surface of the intestinal wall. The large intestine and cloaca may have necrotic foci. Excessive yolk-like fluid is often observed in the abdominal cavity of laying hens.

B. Microlesions.--Microscopic necrotic lesions of the spleen, liver, gall bladder, intestines, and heart often characterize velogenic Newcastle infections. Inflammation and cellular infiltration characterize the serosal lining of the thoracic and abdominal cavities. The hemorrhagic and necrotic lesions of the intestines often consist of lymphoid aggregates. The lesions in the proventriculus are associated with minute ulcerative changes. Lymphocytic infiltration has been described in the pancreas.

IV. Diagnosis:

A. In the field.--The signs of VVND and course of the disease closely resemble those of a number of avian diseases including fowl plague, laryngotracheitis, and the diphtheritic form of fowl pox. Laboratory confirmation of a diagnosis is therefore mandatory.

B. Laboratory.--The surest method of diagnosing VVND or ND is the isolation and identification of the causative virus. Specimens for attempting viral isolation should be selected from cases in the early or even the prodromal stages of the disease. The viral strains causing VVND are widely distributed in the avian body but are present in greater concentration in particular tissues such as the liver, spleen, blood, and lungs. The virus may be isolated by inoculation of these tissue triturates into 9-11 day embryonated chicken eggs. After proper incubation the virus will be found concentrated in the chorioallantoic (CA) fluids. Clear, bacteria-free CA fluid is then tested for avian erythrocyte agglutinating activity. It

should be determined if the reaction is inhibited by known ND antiserums. Identification of NDV is accomplished by means of serum neutralization tests in embryonated chicken eggs, also.

C. Differential diagnosis.--Velogenic viscerotropic ND is likely to be confused with fowl plague (FP). In FP the hemorrhagic lesions on the mucosa of the proventriculus and beneath the proventricular cuticula are much more extensive and severe than in VVND. Virulent NDV kills pigeons in from 3 to 5 days when inoculated intramuscularly. In contrast, the virus of FP, when inoculated into pigeons in the same manner will not cause signs or death. The virus of ND is not neutralized by the antisera from other avian diseases.

V. Prognosis:

In susceptible chickens VVND causes high mortality.

VI. Epizootiology:

A. Geographical distribution.--Velogenic viscerotropic ND has become a menace to the poultry industry. The disease is endemic in India, Indochina, the Philippine Islands, Japan, Korea, Ceylon, Kenya, Egypt, Israel, Syria, and other countries. High mortality has characterized epizootics in these countries. Recently, this type of disease has been reported from countries in various parts of the world. The sporadic outbreaks of VVND in the USA recently were traced to the importation of exotic species of birds (such as parrots and myna birds) from the Orient. These foci of disease were successfully eradicated but not before the disease had spread to commercial poultry flocks in southern California, where the disease was widely spread in several counties.

The disease there was eradicated by the "stamping out" method, disinfection, and decontamination as well as by vaccination. As stated earlier the cost of the campaign in California was approximately 58 million dollars; about 40 million dollars worth of chickens were destroyed.

B. Transmission.--Velogenic viscerotropic ND is transmitted within a susceptible flock by aerosol, contact, contaminated feed, and frequently by people, such as the flock attendants. Visitors are known to have transmitted the disease from one flock to another.

Dissemination of the virus between flocks over long distances has been frequently related to movement of apparently healthy birds in the prodromal or recovery stages. The disease may be transmitted through feeding of infected offal, feed, or water. Contaminated fomites, such as crates, sacks, trucks, etc., may act as mechanical carriers.

The rapid transportation afforded by the airplane has been responsible for spread of the disease. Myna birds shipped from Indochina to Florida and California resulted in outbreaks of VVND.

Virus has been recovered from dressed poultry and this may be another factor responsible for the spread of the disease from one country to another.

C. Hosts.--Domestic fowl and turkeys are the chief hosts although other species of birds are susceptible. During natural outbreaks guinea fowl, ducks, geese, pheasants, partridges, parrots, and myna birds, as well as other flying species, were involved. There is lack of agreement among investigators regarding the susceptibility of wild birds, due to lack of research data regarding many phases of virus-host relationships in these species. However, it is known that most of the gallinaceous

species that are free from antibodies will suffer a lethal infection when inoculated with NDV.

VII. Control and eradication:

A. Preventive measures.--In addition to vaccination, every effort should be exerted to avoid the introduction of the virus to countries which are free from it.

B. Sanitation and disinfection.--A high standard of sanitation should be practiced in management of poultry flocks and on poultry farms. Particular attention should be given to eradication of flies, rodents, and accumulations of feral birds. Upon diagnosis of VVND, the infected premises should be quarantined, the affected flocks and all other birds on the premises should be depopulated and rendered. All the premises should be thoroughly cleaned and disinfected (with a reliable chemical, such as orthophenylphenate). The premises should remain depopulated for at least 30 days before introduction of replacement birds.

The use of sentinel birds on the infected premises for one month before introducing a new flock is useful to indicate the presence or absence of VVND.

C. Treatment.--No drug treatment is known to cure birds from ND infection.

D. Immunization.--Two types of vaccine are available commercially, namely, killed and live virus. These vaccines have been widely used and are very effective when used under favorable or ideal laboratory conditions and according to the manufacturers' specifications. But their efficiency may be modified by conditions in the field, including age, resistance, laying stresses, and sanitary conditions of the flock. In all vaccination

programs the duration of immunity does not exceed several months and may be as short as 3 weeks. Two important aspects regarding vaccination are: (1) There is great variation in protection afforded individual birds by any vaccination; (2) the immunity of individual birds will be affected by the stresses to which they are exposed by management, sanitation, and husbandry of the flock.

VII. Public health aspects:

Newcastle disease virus is capable of causing conjunctivitis in man. Infection is acquired by contact with virus in the laboratory through handling live virus, or in the field by the administering of a live vaccine. Although the majority of human infections are localized in the eye, in a few instances, infection has been accompanied by mild headaches and muscular pains; the virus has been isolated from urine. Laboratory diagnostic workers and others who work with the live virus, including vaccines, should be aware of the possibility of contracting the infection.

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PESTE DES PETITS RUMINANTS *

I. Identification of the disease:

A. Definition.--Peste des petits ruminants (PPR), also known as pseudorinderpest of small ruminants, pest of sheep and goats and kata, is an acute or subacute virus disease of goats and sheep. The acute form is characterized by necrotic stomatitis and intestinal and lymphoid tissue syndromes which resemble those of rinderpest virus (RV) in cattle. The virus is closely related antigenically and immunologically to RV. Cattle are not clinically affected. Goats are more susceptible than sheep and mortality rates in goats vary with activation of latent infection with bacteria or protozoal parasites.

B. Etiology.--The virus is identical to RV in its physical, chemical, and general antigenic properties. The virus particles are spherical in shape and have a diameter of 5,000-7,000A$^{\circ}$ which is larger than RV. The virus particle consists of a core of ribonucleic acid within a fringed outer membrane. Accordingly, it is classified as a member of the paramyxovirus group. The virus has been adapted to multiply in ovine embryo kidney cell culture and induces the formation of large size multinucleated cells with large eosinophilic inclusion bodies. The PPR virus in tissue culture maintained at 40 C retains its pathogenicity for goats and can be used to immunize cattle against experimental RV infection. Tissue culture virus maintained at 37 C rapidly loses its virulence for goats and immunizes this species against virulent virus.

C. History.--Peste des petits ruminants has been reported since 1942 in sheep and goats in some of the ex-French territories of West Africa. The etiological viral agent was isolated and identified in 1956

*Prepared by A. H. Dardiri

as a strain of RV which was naturally adapted to and pathogenic for goats and sheep. It was attenuated for cattle and was believed to lose its ability to infect them by contact. Shortly afterwards extensive new epizootics appeared in Nigeria, causing death among Plateau dwarf goat and sheep. Later the virus was isolated in East Africa from naturally infected goats. Kata is a name used locally in Western Nigeria for pseudo-rinderpest of goats. The disease was first recognized in goats in 1965 and its viral etiology was established in 1968. Goats from Mambilla Plateau in Nigeria were susceptible to experimental infection with either Kata or PPR and disease signs, and gross or histopathological lesions were indistinguishable. The PPR failed to provoke clinical signs of illness in goats that had recovered from Kata. Caprinized RV infections failed to cause clinical reactions in goats previously exposed to Kata and PPR strains. These findings lend support to the conclusion that PPR and Kata viruses and RV are antigenically related.

II. Signs:

A. Clinical features.--The acute form is accompanied by a sudden rise of temperature to 104-106 F. The affected animals appear ill and are restless. They have a dull coat, dry muzzle, depressed appetite, congested mucous membrane, and serous nasal discharge. Ulceration in the buccal cavity sometimes occurs. However, involvement may be limited to severe congestion of the laryngo-pharyngeal mucous membranes. The disease course is relatively short, about 8-10 days, usually resulting in death.

In the subacute form of the disease, which is the form usually seen in experimentally infected animals, no clinical signs of disease

are evident for about 5 days after infection. On about the 6th day, fever and a serous nasal discharge may be found. The fever reaches its peak after 2 or 3 days and then falls after about a week with the onset of diarrhea. In fatal cases, diarrhea becomes progressively more severe, followed by dehydration, emaciation, and prostration. The hind quarters and tail become soiled.

Nasal and lacrimal discharge usually begin as clear serous exudates that later become mucopurulent. On about the 7th to 9th day after inoculation, superficial erosions of the lip and buccal mucosa may be observed. Similar ulcerations or erosions may be found in females on the labial surface of the vulva. The sites of predilection for ulcerations are the lips, gums, buccal papillae, and the ventral surface of the tongue. Stomatitis may or may not be accompanied by salivation. Affected animals may have profuse thick mucoid nasal discharge with coughing and sneezing. Some animals may develop excoriation of the muzzle and commissures of the mouth. Most goats die 6-12 days after the rise in temperature. Some may linger on for 3 weeks after onset of illness. The most frequent complications are secondary bacterial infections resulting in pneumonia and broncho-pneumonia. Pasteurella and Mycoplasma organisms are frequently recovered from such cases. Infection with PPR may activate latent intestinal coccidial infection as well as hematophagous parasites. Abortions occur frequently.

B. Incubation period.--The incubation period in the natural disease may range from 2-15 days.

III. Pathologic changes:

A. Post-mortem lesions.--Animals that die following an acute form of PPR do not exhibit lesions other than congestion of the mucous membranes

and occasional congestion of the ileocecal valve. In some cases, there may be secondary broncho-pneumonia. Lesions in frank clinical cases resemble those of RV infection in cattle, but tend to be less intense. Pulmonary involvement is more frequent than in RV infections. The carcass is emaciated, soiled, fetid, and the nostrils are encrusted by a purulent nasal discharge. The lips are hyperemic. Lesions in the mouth vary from a few erosions of the mucosa of the soft palate to extensive necrotic ulcerative stomatitis. Erosions may extend into the congested pharynx. The mucosa of the abomasum may show a diffuse congestion. Severe congestion may extend throughout the alimentary tract but, more often, changes are limited to the duodenum, ileum, cecum, and the upper colon. The mucosa of the ileocecal valve is a prominent site of congestion and sometimes there may be hemorrhage. The crests of the longitudinal folds of the cecum, colon, and rectum are sometimes congested giving the appearance of "zebra" stripes.

The mucosal lining of the upper respiratory tract and trachea is generally congested. Patches of congestion in the lungs is common, and on occasion, broncho-pneumonia lesions will affect the apical and cardiac lobes.

Usually the heart appears grossly normal. Sometimes, petechiae are present near the coronary vessels. Most usually, the mesenteric lymph nodes are edematous. The spleen usually is grossly normal, but occasionally it is swollen slightly and the capsule is injected. Congestion and erosion is common in the large Peyer's patches of the terminal part of the ileum. There are no specific liver lesions.

The mucous membrane of the urinary bladder is normal or slightly congested. Kidney congestion and erosion of the vulvar and vaginal mucosa occur occasionally.

B. Microlesions.--The necrosis of the mucosa of the oral cavity is marked by the presence of intranuclear and intracytoplasmic inclusion bodies and occasional syncytia in the stratified epithelium as in RP. Intranuclear inclusion bodies are found in the reticulo-endothelial cells close to the sinus and germinal centers of the lymph nodes.

IV. Diagnosis*:

A. In the field.--A presumptive diagnosis of PPR may be made when there is a new epizootic in goats and sheep which is accompanied by mortality and disease signs and lesions as described for PPR. However, because some of these signs or lesions are common to other sheep and goat diseases, a laboratory confirmatory diagnosis is necessary, which is achieved by isolation and identification of the virus.

B. Laboratory.--Blood, spleen, and mesenteric lymph nodes from sick animals or those in extremis are the tissues of choice for submission to the laboratory. Serums from recovered animals are necessary for the detection of PPR antibodies. Among the tests, which may be performed in the laboratory, is the demonstration of cross-protection of goat and sheep infected with PPR and RV. Susceptible goats exhibit PPR disease signs upon inoculation with the virus. Cattle resist challenge with the virulent RV subsequent to their inoculation with PPR virus.

The PPR virus can be isolated in cell culture and identified using serological tests such as virus neutralizing and complement-fixation tests.

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*See also appendices 1 and 2.

Antibodies in the serums from animals which recovered from PPR may be detected and assayed by the same serological tests.

C. Differential diagnosis.--Many types of secondary pathogens are involved; however, the more common are protozoa, either in the circulatory or the intestinal systems. Dual infection yields complex clinical signs and postmortem lesions. Often the resulting disease features are dominated by those attributed to activated infections. Diseases such as bluetongue, coccidiosis, Nairobi sheep disease, contagious ecthyma, plant and mineral poisoning have clinical and pathological manifestations similar to those of PPR. The diagnosis of bluetongue requires serological confirmation. The only certain way to diagnose coccidiosis is to find active lesions containing coccidia at necropsy. Sometimes the history of an outbreak may help differentiate PPR from plant and mineral poisoning; however, serological tests are necessary. Mice inoculated with blood from sheep with Nairobi sheep disease die, but they will remain healthy if they are infected with PPR.

The buccal lesions around the mouth and nasal openings associated with PPR can be confused with those of contagious ecthyma and pox. Therefore, laboratory confirmation is necessary for disease differentiation. Inoculation of susceptible animals and histopathological examination will assist in diagnosis. In sheep and goat pox, papules, vesicles, and scab formation are generally more extensive on the body. Contagious ecthyma produces proliferative type lesions and can be distinguished by cross-immunity tests.

V. Prognosis:

Mortality in goats range from 10-90%. Sheep are less susceptible than goats and rate of recovery is significantly higher than in goats. The mortality rate is affected by the animal's condition, its innate resistance, the virulence of the virus, and secondary complications caused by activation of latent infection in the animal.

VI. Epizootiology:

A. Geographical distribution: PPR is an enzootic disease in West French Africa. It was reported mainly in the southern and central regions of these countries and rarely in the northern regions. The disease exists in Nigeria, the Ivory Coast, Senegal, and Dahomey.

B. Transmission.--Infection may be transmitted to susceptible animals by direct contact with infected animals or indirectly by contact with secretions and excretions. Movement of infected animals may be the chief factor in the spread of the disease. All tissues and fluids from infected animals should be considered infectious throughout the period of clinical illness.

C. Hosts.--PPR is an enzootic disease of goats and sheep in West Africa. Cattle develop serological response without clinical signs as a result of inoculation. Infection of cattle by contact has not been conclusively proven.

In spite of the close resemblance of PPR disease signs, lesions and the viral properties to RV, cattle exposed to PPR do not have any clinical reaction but do acquire a solid immunity to RV.

VI. Control and eradication:

A. Preventive measures.--When PPR is suspected, state and federal veterinarians should be notified. Methods that are applied to RV eradication are useful in eradication and control of PPR. The stamping out procedure is recommended when the disease appears in new areas. All sick goats and sheep and those in contact should be slaughtered and disposed of by burning, burying, or rendering. Infected premises should be decontaminated and the area should be quarantined. Import restrictions on sheep and goats from countries where the disease is enzootic should be applied. Movement of animals and their products from suspected premises should be prevented. Epizootic surveillance of sheep, goats, and cattle should also be undertaken to determine the extent of disease present or its dissemination.

B. Treatment.--There is no specific treatment for PPR. However, administration of products which alleviate bacterial and parasitic complications decrease the mortality of affected herds.

C. Immunization.--Susceptible goats have been protected from infection with PPR by inoculation with serum from cattle which were hyperimmunized against RV. Limited success in protection of susceptible animals was obtained by using inactivated and lapinized RV vaccines. More recently, the use of cell culture RV vaccine has proved effective in the immunization of susceptible goats against exposure to natural infection or experimental immunity challenged with PPR virus. In addition, an attenuated vaccine was obtained by 51 passages of PPR virus in embryo kidney cell culture. This vaccine is very efficient in protecting goats and sheep from natural disease for about a year.

VII. Public health aspects:

Man is not susceptible to RV and it is believed that PPR is not communicable to human beings. However, serum PPR convalescent goats inhibit measles virus in the hemagglutination inhibition test.

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APPENDIX 1

SUMMARY OF CHARACTERISTICS OF PESTE DES PETITS RUMINANTS (PPR)

Description	Cause and Distribution	Host	Pathology	Diagnosis
An acute, sub-acute or chronic disease of goats and sheep with rinderpest-like lesions in cattle. Necrotic ulcerative stomatitis, pneumonia and diarrhea.	Virus. Ex-French territories in Agrica.	Goat, sheep and cattle.	Ulcerative necrotic stomatitis. Congestion in the alimentary tract, diarrhea and emaciation.	History, signs and lesions, virus isolation and identification. Gross protection tests in goats and cattle.

Incubation Period	Mode of Transmission	Period of Communicability	Control Measures
6-15 days.	Direct contact with sick animals. Contamination of premises by secretions of sick animals.	During incubation period and disease course.	Stamping out and quarantine measures. Use of vaccine in enzootic areas.

APPENDIX 2

DIAGNOSTIC TESTS FOR PESTE DES PETITS RUMINANTS (PPR)

Microbiological Procedures	Histological Examination	Serological Tests	Animal Inoculation
Virus isolated from spleen, blood and lymph nodes	Nasal catarrh, ulcerative and necrotic stomatitis, pneumonia and congestion of alimentary tract	Virus neutralization and complement fixation tests	Susceptible goat, sheep and cattle

PIROPLASMS AND PIROPLASMOSIS*

These are parasitic protozoa which inhabit the erythrocytes and sometimes other cells of vertebrates, and which do not form pigment from hemoglobin (as do malarial organisms). They cause a group of diseases known, generally, as piroplasmosis. Piroplasms are small usually round or pearshaped when in the erythrocyte and are found in fish, amphibian birds and mammals. As far as it is known they are all transmitted by ticks—the method of transmission in aquatic hosts is not known.

The most important organisms in this gruop are the genera Babesia,[1] Theileria,[1] Haematoxenus,[1] Cytauxzoon,[2] and Dactylosoma.[2] Babesia parasitize reptiles and birds but mainly mammals; Theileria Haematoxenus and Cytauxzoon parasitize mammals and Dactylosoma cold-blooded vertebrates.

Certain Babesia and Dactylosoma species are endemic to the USA; Haematoxenus is confined to cattle in Africa; Cytauxzoon has recently been identified in the USA,[5] however, it is the Theileria that are very prevalent throughout the world, are often very pathogenic, and constitute a potential threat to US livestock and mammals.

THEILERIASIS

I. Identification of the disease:

A. Definition.--Theileriasis is a disease of domestic animals in which the protozoan parasite infects the erythrocytes and lymphoid cells. The disease varies from mild to an acute febrile and often fatal condition; an extremely severe form is seen in cattle in East Africa caused by Theileria parva and known as East Coast Fever.[1,3]

*Prepared by C.M. Groocock

The important species of this genus are listed in Table I.

B. Structure.--The erythrocytic forms are very pleomorphic and appear as rods, rings, and oval forms; they are 1 to 2 long by 0.5 to 1 wide. The exoerythrocytic schizonts found mainly in the spleen and lymph nodes are irregularly oval in shape and measure 10-20 in diameter. These are a mass of cytoplasm containing many nuclei; the whole schizont is referred to as a Koch's Blue Body and its detection is diagnostic of theileriasis.

C. Etiology.--The vectors, in all known cases, are ixodid ticks and the infection can pass from stage to stage of the tick (transtadial) but not through the tick's eggs (transovarial). The tick becomes infected by ingestion of the erythrocytic forms. The development undergone in the tick is little known, however, after necessary development, infective forms appear in the saliva of the tick. Following attachment of the tick on a susceptible host, the infective form is injected with the saliva and migrates to the draining lymphnode, where it undergoes schizogony. Several generations of macroschizonts followed by microschizonts occur and the latter produce forms that infect the erythrocytes.

II. Signs:

A. Clinical features.--The first sign is enlargement of the draining lymphnode followed by fever (105-106°F). Clinical signs are not severe during the early stages. Later there is generalized lymphadenopathy and deterioration of the animal with dyspnea, congestion of mucous membranes and sometimes anemia. Froth is often present in the respiratory tract and nostrils at time of death. The duration of the disease may be from 4 to 25 days. Morbidity may be 95% and mortality 100%.

III. Pathologic changes:

A. Postmortem lesions.--There is widespread edema of the carcass with a gelatinous appearance of the subcutis and muscle fascia. Serous and mucous membranes may be covered with petechial hemorrhages. There is generalized hymphatic hyperplasia and apparent liver and kidney infarcts of perivascular lymphocytic foci.

B. Microscopic pathology.--An intense lymphocytic hyperplasia is seen early in the disease. This is followed by depletion and destruction of the lymphocytopoietic centers.

IV. Diagnosis:

A. Field.--A febrile disease associated with lymphadenopathy, pulmonary edema, and an appropriate tick infestation are only indicative of theileriasis. Further tests are needed to confirm the infection.

B. Laboratory.--The demonstration of Koch's Blue Bodies by lymphnode biopsy, and of piroplasms in giemsa-strained blood smears is diagnostic of theileriasis. Serological tests are not fully reliable because of non-specific cross reactions, though they can be used to augment a provisional diagnosis.

V. Control:

A. Preventive measures.--Dipping or spraying with acaricides to control tick infestation. Weekly applications are usually sufficient however, in endemic East Coast Fever areas, three-day intervals may be required.

B. Treatment.--No effective treatment exists once clinical signs are well established. However, some experimental drugs show promise and early treatment, during incubation, with tetracyclines at the rate of

5-15 mg/kg given daily may suppress schizogony and moderate the pathogenesis.

C. Immunization.--No commercial vaccines exist because of the wide spectrum of anitgenic strains. Concomitant induced infection and treatment with tetracyclines has been successful in immunizing against homologous strains. Recovery usually results in an immunity which may persist for many years, however a state of preimmunity or recurrent challenge may be required to maintain the immune state.

TABLE 1—Theileriidae of Veterinary Importance

Species	Geographic distribution	Main hosts		Patho-genicity	Common name of disease (if any)
		Vertebrate	Invertebrate		
Theileria parva	East Africa (eradicated from Southern Africa)	Cattle	Rhipicephalus Hyalomma	High	East Coast Fever
T. annulata	N. Africa, S. Europe, S. Russia, India, China	Cattle, water buffalo	Hyalomma	High	Mediterranean Coast Fever
T. mutans	Africa, Asia, Europe (including England), Russia, Australia, N. America	Cattle	Rhipicephalus Boophilus	Very low	
T. hirci	N. Africa, S.E. Europe, S. Russia, Middle East	Sheep, goat	? Rhipicephalus	High	
T. ovis	Africa, Europe, Russia, India, Middle East	Sheep, goat	Rhipicephalus	Very low	
T. cervi	United States[8]	White tailed deer	?	Moderate	
C. felis (provisional)	United States	Cat	Unknown	High	

TRYPANOSOMES AND TRYPANOSOMIASIS

These are elongate flagellated protozoa which are all parasitic and several are important pathogens. Three genera are parasites of vertebrates Leishmania, Trypanosoma and Endotrypanum. Many of the species of these genera also parasitize blood sucking invertebrates, usually insects or leeches, by means of which they are transmitted from one vertebrate to another. These organisms are highly successful and are found worldwide in many species (Levine 1). Trypanosomes are common parasites of fishes, amphibia, reptiles and birds in addition to their extensive parasitization of mammals. The latter group include all the most important pathogens.

AMERICAN TRYPANOSOMIASIS (Chagas' Disease)

American trypanosomiasis is mainly a parasitic infection of man causing severe and widespread disease in South and Central America. A wide range of wild animals have been reported to be infected with this organism and its isolation and identification in rodents and racoons have been reported as far north as Maryland in the United States. However these infections appear to be nonpathogenic and very few autochthonous cases in man and animals have been reported. The vectors are bugs, in a strict sense, of the family Reduviidae. There is no known treatment. A full description of the infection is given by Bray.[6]

AFRICAN TRYPANOSOMIASIS

I. Identification of the disease:

Definition.--African trypanosomiasis is a parasitic disease of man and animals caused by a grup of related trypanosomes that are trans-

mitted by Glossina spp. (tse-tse flies); the few exceptions have evolved drom forms which had a tse-tse vector. These parasites inhabit the blood plasma and body fluids. Some are highly pathogenic causing severe disease in domestic and wild animals and "Sleeping Sickness" of man.

B. Structure.--These are curved "eel like" organisms which are pleomorphic with slender to stumpy forms. They are 12 to 42 long with or without an anterior flagellum or whip. They all have an undulating membrane running the length of their body (like the dorsal fin of an eel). They have a central nucleus and, posteriorly, a large mitochondrion containing DNA, from near which the flagellum and undulating membrane arise.

C. Etiology.--Trypanosoma congolense infects most domestic and many wild animals, T. vivax domestic and wild ruminants, and T. brucei-evansi group can be found in nearly all domestic and a great number of wild animal species.

II. Signs:

A. Clinical signs.--Trypanosomiasis is often a chronic disease extending over weeks or months and usually results in death if untreated. The signs are varied and many. As the disease progresses emaciation, anemia and cardiac malfunction are commonly seen. Central nervous signs (hence sleeping sickness) are usually a sequel of T. brucei group infections. Highly pathogenic episodes occur in cattle infected with T. congolense with onset of fever, anorexia, dullness and photophobia. Anemia and shallow respiration are obvious signs. The spleen becomes enlarged and the heart decompensated with venous congestion and edema.

B. Incubation period.--Highly variable depending upon strain and species of parasite and upon host, 4-28 days.

III. Pathologic changes:

A. Pathogenesis.--The trypanosomes replicating in the blood and tissue spaces produce copious amounts of metabolites among which a toxin has been identified. There is massive degeneration of collagen fibres with resultant destruction of small blood vessels leading to tissue anorexia and edema. The choroid plexus is severely affected in T. brucei infections. In the early stages the toxin causes a hemolytic anemia, later there occurs chronic bone marrow depression and failure of iron metabolism.

B. Blood changes.--There may be a 50% decrease in erythrocyte count as if hemorrhage had occurred.

C. Post mortem.--Lesions are variable and in acute cases generalized signs of systemic infections are seen with extravasation into the body cavities, petechial hemorrhages on the membranes and myocarditis. The chronically infected carcass is emaciated and edematous; specific organ damage is manifested by a myocarditis, adrenal adenitis and orchitis. The liver, spleen and lymphnodes are congested and enlarged. The pathology of trypanosome infecti-ns has been extensively reviewed by Lossos.[7]

IV. Diagnosis:

A. Field.--As emphasized above clinical signs are variable. Appropriate disease signs in a known endemic area and presence of vectors requires trypanosomiasis to be excluded or confirmed by laboratory tests.

B. Laboratory.--Diagnosis is confirmed by the demonstration of trypanosomes in Giemsa stained peripheral blood and lymph node biopsy smears.

All trypanosomes spp. are not necessarily found in the blood some, such as T. brucei, are mainly tissue dwellers. A negative smear should be treated with caution for, late in the disease, organisms become scarce and thick blood smears taken on consecutive days should be examined.

V. Prognosis:

The outcome is usually fatal however these diseases respond well to drug treatment and spectacular cures can be achieved if treatment is initiated early enough.

VI. Control:

A. Preventive measures.--Attempts to eradicate the vectors are expensive and ethically questionable as they involve widespread dissemination of insecticides and have involved the wholesale destruction of wildlife. Initial successes in Southern Africa in the 1950's have been marred by re-invasion of clean areas by the tse-tse fly.

B. Treatment.--A series of trypanocidal drugs are available based on quinopyrimines, diamidines and phenanthridines. Good, long lasting, protection is achieved by Suramin (quinopyrimine) however side reactions can be severe.

C. Immunization.--The possibility of vaccination is, at present, remote. These organisms undergo rapid and repeated antigenic shifts. Little immunity is conferred by one antigenic type against another type.

VII. Epizootiology:

A. Geographical distribution.--Approximately two-thirds of tropical Africa is infected by the tse-tse fly; as a consequence economic animal farming and reasonable animal protein production is denied to an area greater than the contiguous United States. Trypanosomiasis (T. evansi

group) extends beyond this area into Middle and Far East Asia, Central and South America. T. vivax normally transmitted by the tse-tse fly has established itself in South America and the West Indies.

B. Transmission:

1. Source of infection.--Carrier domestic stock and wild animals are the reservoirs of trypanosomes. The tse-tse fly is the principle vector though otherbiting flies are efficient diseminators of the organisms.

2. Cyclical transmission.--T. congolense, T. vivax, T. simiae (pig), T. brucei in addition to T. gamsiense, and T. rhodesiense (himan sleeping sickness) are all transmitted by Glossina spp. in which they undergo development and multiplication.

3. Mechanical transmission.--During interrupted blood meals by biting flies (Tabanids and Stomoxys spp.) infected material can be transferred between hosts. This method is surprisingly efficient resulting in the spread of T. evansi throughout Asia and South America and T. vivax to the western hemisphere.

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RINDERPEST*

Identification of the disease:

Definition.--Rinderpest (RP), from the German, was previously called cattle plague. The disease is currently also known as peste bovine, peste bovina and pestis bovina (FAO Animal Health Yearbook). Rinderpest is an acute febrile, highly contagious and often fatal disease of ruminants, particularly cattle and buffaloes. The virus may be found in all organs of the infected animal, but has an affinity for the mucosa of the gastrointestinal tract and the lymphatic tissues. At the cellular level the virus has been shown to attach to the leukocytes.[53]

Etiology:

Classification.--In 1848 Holmes Placed RPV in the genus Tortor because of its tissue tropism range. Later it was subgrouped with measles and canine distemper viruses ("MRD") because of the large syncytial aggregates associated with cytopathic effect (CPE) in cell cultures and the close immunological relationship of RP, canine distemper and measles viruses. The virions of each of the 3 viruses haveidentical structures similar to that of Newcastle disease and other larger myxoviruses.[55] The provisional committee for nomenclatures of viruses later [1] proposed paramyxoviridae as the family name for this group.

The majority of RPV particles are spherical or ovoid with a maximum diameter of 1200-3000A^{o}, in highly centrifuged and highly concentrated preparations; some having more irregular outlines, are as large as 7500 A^{o}. Details of the fine structure of RPV, as revealed in electron micrographs, were reported by Plowright et al.[2] Most particles have an outer membrane which is often fringed with short projections. Internally, there is a

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tightly coiled component approximately 18 nm in diameter with easily resolved serrations 5-6 n. apart. Fenner [10] classified RPV in the paramyxoviridae family because of its single stranded linear RNA and molecular weight of $5X10^6$. He likewise placed RPV in the genus Morbillivirus with measles, canine distemper and peste des petits ruminants (PPR).

Physiochemical and Immunologic Properties.--All efforts to demonstrate hemagglutinins for RPV have failed so far. However, RP antisera inhibits measles hemagglutinin. All strains of RPV show immunological homogenicity. Rinderpest virus antigen can be detected by several serological tests such as complement fixation (CF), agar gel diffusion precipitin (AGDP), fluorescent antibody (FA) and virus neutralization (VN).

Thin layers of rinderpest-infected blood are inactivated by 2 hours exposure to sunlight. Tissue culture propagated virus survives heating at 56°C for 50-60 minutes and exposure to 60°C for 30 minutes.[36,7]

Stocks of cultured virus which are frozen at -25 and -70C can be maintained with a fall in titer for over a period of 17-23 weeks. Frequent freezing and thawing of blood samples causes inactivation of RPV.

Freeze-dried cell culture viral vaccine stored at -20°C does not decrease in titer during a 4-6 year period. Virus lyophilized with a lactalbumin sucrose additive is stable at 4°C for over a period of 2-1/2 years. The optimal pH range of RPV is 6.5-7.0. Inactivation is very rapid at pH 2.0 and at pH 12.0 takes 10 minutes. Glycerol inactivates rinderpest virus.

Lipid solvents, such as 20% volume of either of 5% chloroform inactivate the virus in 10 minutes at 22°C. Suspensions of bovine or lapine spleens containing $10^{4.7}$ lethal doses of RPV per ml are inactivated within 30 minutes by treatment with 0.4% B-propiolactone (BPL) at 28°C. Furthermore, exposure of RPV to 0.1% BPL at 4°C for 18 hours also completely inactivates its infectivity.

Host cell range.--Rinderpest virus grows readily in monolayers of epithelial or fibroblastic cells from many animal species including ruminants, swine, dog, hamster, chick embryos and monkeys. The cytopathogenic effect (CPE) of RPV is characterized by the formation of multinucleated giant cells (syncytia). Infected cells frequently contain eosinophillic cytoplasmic and intranuclear inclusions.

(1) Natural host range:

a. Domestic animals.--Cattle and water buffaloes are the most important natural hosts of RPV; but, natural outbreaks of the disease have been observed in goats.[4,7,49] In addition, serological evidence of extensive subclinical infection in goats has been obtained in several East African bountries.[59] Many sheep in Nigeria in the vicinity of rinderpest outbreaks of the disease in cattle also had antibodies.[59] Camels can be infected experimentally by the parenteral route and the disease can spread from cattle to camels by close contact.[49] All species of the order Artiodactyla are sysceptible to natural infection with RPV. Yaks in zoological gardens died from rinderpest.[3] Domestic swine in southeast Asia are susceptible in European type pigs by feeding infected material or by contact with infected cattle.[45,47]

b. Wild animals.--Natural infection has been proven in 8 species of free living wild animals: the wart hog, giraffe, kudu, eland, African buffalo, wildebeest, impala and Thomson's gazelle. The following species have been infected in captivity: the wild pig, sumbar, bushbuck, sitatunga, reedbuck, blackbuck oribi. Artiodactyles experimentally infected with rinderpest were bushpig, Japanese deer, guar, duiker, water buck, kob, oryx and dick-dick.

Alleged but unproven infections incriminate 27 more species of artiodactyles. Among these were peccaries which died following experimental administration of rinderpest virus. It may be generalized that of all the species of African game animals, the buffalo, eland, wildebeest, wart hog, bushpig, kudu and giraffe are regarded as most susceptible.

(2) Experimental host range: The first attenuated virus for use as vaccines were prepared in goats. Goats of different breeds or from different localities were reported to vary considerably in their response to inoculation with goat-adapted virus;[48] some animals appeared to be completely resistant.

The most important adaptation of RPV to rabbits was carried out by Nakamura and his colleagues in 1938. They also reported that rabbits could be infected by intravenous or subcutaneous inoculation of bovine blood infected with virulent rinderpest virus. Several workers adapted the rabbit-passaged viruses with a view to their utilization as attenuated virus vaccines. In addition, embryonated eggs proved to be useful indicator hosts in serum-virus neutralization tests.[25]

The adaptation of the Kabete "O" strain of the virus to cell culture[33] is a recent advance in the study of rinderpest. Attenuated cell culture adapted RPV has great potential as an economical vaccine for field use.

History:

Rinderpest is an ancient disease of the bovidae and was described as early as the fourth century. Its wide spread in Europe was associated with major military campaigns. Later in the 19th century, establishment of railroads and canals enhanced the role of international cattle trades as a disseminator of infection. Since then, the cource of virgin epizootics is traced to importation and movement of bovidae.

Rinderpest reportedly came to Europe from the East, allegedly from the Caspian Basin or the Russian and Hungarian Steppes, and extended along the Valley of the Danube. About 200 million cattle are estimated to have died in Europe in the 18th century. Thereafter, wide dissemination of the disease led to the 1865 catastrophic outbreak in Great Britain. This continuing spread of RP was an important factor in the establishment of the first veterinary schools in Europe beginning with Lyon in 1762. Likewise, these events stimulated the decision to convene the first international veterinary congress in Hamburg in 1863. More recently RP was responsible in large measure for the foundation of the Office Internationale des Epizooties in Paris,[58] following in 1920 the introduction of the disease to Belgium by cattle transshipped at Antwerp en route from India to Brazil.

By 1930, with the exception of parts of Turkey, Europe was free from RP. Since then confined and small foci of infection have occurred in Rome[3] and Trieste.[2] Rinderpest, however, continued to persist in the Indian subcontinent.

The only outbreak recorded in the Western Hemisphere had its origin in Indian cattle shipped into Brazil in 1920.[40] An outbreak occurred in Australia in 1923.[43,58]

The infection repeatedly gained access to the Nile Valley via Europe and eventually spread to the Sudan and West Africa. The most recent and spectacular epizootic in this part of Africa was introduced by Indian cattle to Somaliland[14] during the Italian invasion of Ethiopia in 1889. Thereafter the disease spread to Uganda, Kenya, Tchad, Cameron, Nigeria, the Niger, Volta, Mali, Mauritania, Senegal, Gambia, Ivory Coast, Ghana, Togo, Dahomy and Siera Leone.[46] Rinderpest was eliminated from the Cape Colony by 1903 and soon afterwards from the rest of South Africa. It is still enzootic in East, Northeast, Equatorial and West Africa.

The filterability of the causal agent was demonstrated in 1902.

Sites of rinderpest virus replication in cattle:

Cattle can be infected experimentally with RPV by any route of inoculation. Infection takes place readily by use of nasal discharges administered by conjunctival instillation.[43] The virus is regarded as incapable of passing through the intact skin of ruminants, but pigs appear to be easily infected by eating contaminated food which results in a dominant role in disease dissemination.

Infection takes place readily in the upper respiratory tract by nasal swabbing with excretions of infected animals or by nasal instillation of virus. Following intranasal instillation the virus is demonstrated after 24-48 hours in either or both mandibular and pharyngeal lymph nodes as well as in the palatal tonsils. Viremia is detected from the second to the third day onwards, i.e. 1 or 2 days before the appearance of pyrexia. It has been

concluded that natural infection takes place invariably via the upper respiratory tract and sometimes via the lower respiratory tract. It was theorized that RPV is carried through respiratory mucosa without proliferation. Mauer et al.[22] stressed that RPV has a selective affinity for lymphocytes and epithelium of the alimentary tract. It is agreed[16,17,22] that multiplication of the virus causes almost complete lymphocytolysis and produces erosions and ulcers in the mucosa of the alimentary tract through necrosis of the epithelial cells. In addition, multinucleated giant cells, or syncytia, are found in eosinophilic intracytoplasmic inclusions in lymphoid tissues and in the stratum spinonsa of the stratified aquasums epithelia of the upper alimentary tract. Intranuclear inclusion bodies are observed. Rinderpest virus can be detected in most if not all tissues during fever.

Incubation period:

The incubation period of RPV is dependent upon the strain dose administered and route of administration. In general, the experimental incubation period lasts for 2-9 days. The incubation period following contact exposure range is usually 4-5 days; however, some strains require 10-15 days.

Virus appears in the blood 1-2 days before the onset of fever,[30] and is generalized throughout the body by the end of the incubation period. Between the time of onset of pyrexia and the first appearance of mucosal lesions in the mouth (in an average time of 3 days), the virus is found in high titer in the lymphopoietic tissues and also in the mucosa of the alimentary tract. The virus may also be found in high levels in the nasal mucosa, and lungs, but at lesser levels in the liver. During this period,

the virus in the lymphoid tissue and abomasal mucosa reached the following mean level:[43] lymphoid tissues and abomasal mucosa $10^{6.0}$ ID_{50} 1 ml blood $10^{4.5}$ ID_{50} 1 ml; bone marrow $10^{1.0}$ -$10^{2.0}$. In cattle infected with mild field strains recently isolated in East Africa[31], the peak mean blood titer measured in tissue culture reached only $10^{1.94}$ on the fourth day in the prodromal period. The distribution of virus in various animal tissues is a function of the cytopathogenic potential of the virus strains. The virulent strains multiplied to a high titer in the mucosa of the respiratory and alimentary tracts, whereas, the attenuated strain cell culture was apathogenic and could not be isolated from these tissues.[31]

During the mucosal and convalescent period which are characterized by mouth lesions and diarrhea, the virus began to decline rapidly. The first appearance of antibody occurred on the fifth or sixth day of the disease caused by virulent viruses. The lymphatic tissue in which the virus first proliferates is the tissue in which the decline in titer first begins. The alimentary mucosa, respiratory tract tissues and lympho-epithelial structures, such as the tonsils, retained viruses longest.

Carrier state.--The persistence of RPV in the blood and other tissues of cattle with circulating antibody is explained[30] by its association with leukocytes. The leukocyte fraction contains traces of virus after convalescence.

Excretion of virus by infected cattle:

Nasal excretion of virus occurs 1-2 days before fever and then declines by the ninth day. Fecal excretion is considered one of the main disseminators of virus. The conjunctival and oral secretions are infective. Milk from recovered animals is infective for up to 45 days.

The nasal excretion of virus first became detectable 1-2 days before fever, but the nasal excretion rate rose gradually to a maximum of 87.5% on the fourth day of fever. The quantity of virus was 10^4 -10^5 ID_{50} between the third and seventh day of fever, but declined thereafter so that it was not found after the ninth day.

Urinary excretion rate rose to about 62.5% on the seventh day of illness.

Fecal excretion[19] is considered one of the main methods of dissemination of the virus. The maximum fecal titer varied between 10^4 -10^6.

The conjunctival and oral secretions of adult animals and vaginal exudates from aborted femailes are infective. Milk also contains virus which is said to persist in recovered animals for up to 45 days.[5]

Immunological variation of RPV strains.--All RPV strains are immunologically very similar. The most important variations which occur in RPV strains are in the host afinity, pathogenicity and transmissibility.

Subclinical infections may occur in some animal species and hence serological examination is necessary for detection of exposure. The degree of exposure may vary considerably due to methods of feeding and watering, as well as concentration of population.

In East Africa, the virus has been known to affect sheep without spreading to goats[42] and goats without apparently involving sheep.[17] In India, the disease in sheep and goats did not always spread to cattle.[47]

Mild strains:

A strain of RPV was isolated from a sick eland shot in Tanganyika.[40] The isolate was not virulent for cattle and passage in cattle did not increase its pathogenicity.[20] Seven other strains recently isolated in East Africa

proved non-lethal to cattle.[27] A related virus, PPRV[23] killed sheep and goats but was avirulent for cattle. A recent outbreak of RP in Nigeria was characterized by high morbidity and mortality in both sheep and cattle.[14]

Pathogenicity and transmissibility:

The considerable genetic variation in susceptibility of domestic animals makes the assessment of pathogenicity of RP difficult. Naturally[5] occurring strains of RP appear to fall into two main types as far as their pathogenicity in cattle is concerned. Those isolated in South Kenya and North Tanzania have invariably produced mild disease in cross bred Zebo-exotic animals with low mortality in uncomplicated cases. These strains produced typical mouth lesions in 70% of the cattle and diarrhea in about 20%; but spread rapidly by contact.

In Northeast Africa, RPV of higher pathogenicity to cattle and lethal to a large number of game animals[54] was isolated.

It is a mistake to regard the majority of RPV strains as being easily and consistently transmissible by natural routes even if the donor animals are in their most infectious state. The stock laboratory strain of Kabete "O" virus produced 55% mortality when it was inoculated subcutaneously; however, it did not spread readily by contact nor did it produce any characteristic mouth lesions.[26]

Caprinized, lapinized and avianized strains which have been employed as live vaccines do not spread by contact. Similarly, cell culture attenuated virus is not known to spread from inoculated cattle to other cattle in close contact.[54]

Immunity to rinderpest virus:

Infection with virulent strains of RPV confers a solid and life-long immunity to parenteral and natural contact challenge. Cattle inoculated with a virulent or culture virus develop antibodies for at least 6-7 years.

The time of appearance of neutralizing antibodies in cattle was found to be the fifth or sixth day post infection in animals inoculated with large doses of the virulent Kabete "O" strain.[29] Culture attenuated virus stimulated antibody formation on the sixth day after inoculation of $10^{4.5}$ $TCID_{50}$. Development of neutralizing antibodies seemed dependent upon the virus concentration in the inoculum. Neutralizing antibodies appeared from the seventh to the seventeenth day with decreasing virus doses. Some cattle given small doses of culture attenuated RPV did not develop antibody by the end of the second or third week but they were immune to challenge.[29] Peak neutralizing antibodies were usually reached between 2 and 4 weeks after infection. A small decline in antibody titers was detected during a period of 8 to 18 months as well as 2 to 4 years.[14]

Soluble antigens and related antibodies:

Complement fixing antigen.--Complement fixing (CF) antigen is first detected on the onset of fever in lymph nodes and reaches its peak titer within 48 hours. The decline coincides with the fall in temperature. No antigen can be detected 11-12 days after the onset of fever. Complement fixing antibodies appear in the serums of cattle on the ninth to seventeenth day. They reach a peak in 14 to 18 days and remain demonstrable in some cases for as long as 6 months. The CF antibodies are not a

reliable guide to measure response to immunization or epizootiological surveillance because of their short lived character.

Precipitinogen antigen.—Diffuse precipitinogen antigen in the prescapular or mandibular lymph nodes of cattle with a virulent strain of RP obtained by biopsy samples is detected on the first to eight day after the onset of fever. The highest percentage of positive samples occurs on the third to fifth day coinciding with the onset of diarrhea.

Antisera for use in agar diffusion tests are prepared by the hyperimmunization of cattle or goats. Precipitating antibodies cannot be demonstrated in the serum of convalescent animals.[57] An indirect gel diffusion test is used for demonstration of antibodies in convalescent sera; however, more work is needed to develop its full potential.

Antibody inhibiting measles hemagglutinin.--Infection of cattle with virulent (or attenuated) RPV stimulates antibodies which inhibit measles hemagglutinins to high titers. These antibodies appear 9 to 12 days after infection with virulent RPV. The titer depends, as with CF antibodies, on the virulence of the infecting virus. It remains detectable for several months but later declines to non-detectable levels in some animals. The test is therefore not suitable for serological surveillance of RP.

Immunological relationship between rinderpest and other viruses:

Rinderpest and measles.--Neutralizing antibodies are present in serums of humans and monkeys during convalescence from measles.[9] Monkeys inoculated with RPV show mild febrile reactions and low levels of neutralizing antibodies. They are not protected against challenge

with measles virus. The sera of RP-immune cattle contain antibodies to measles virus. A single dose of virulent measles virus ($10^{3.6}$ or $10^{6.4}$ $TCID_{50}$) fails to protect cattle against challenge with RPV and no antibody is produced to either agent.[9] Rabbits are not protected against challenge with lapinized RPV by a single dose of measles virus. However, three or more inoculations by various routes cause production of RPV neutralizing antibody and protect the rabbits against challenge with lapinized RPV.[27]

Rinderpest and canine distemper.--Dogs inoculated with virulent RPV or lapinized RPV develop viremia and antibodies to RPV but not to distemper. Rabbits which receive virulent avianized canine distemper (CD) virus are not protected against challenge with lapinized RPV. The resistance to RPV in cattle conferred by CDV is controversial. It was reported that protection of cattle was correlated with the production of antibodies to CDV.[24] However, it was found[7,9] that virulent CDV did not protect cattle against RPV although CDV antibodies were found. Rinderpest antisera confer no passive protection when administered simultaneously with virulent CDV either to dogs or ferrets.[13]

Rinderpest clinical disease features:

Clinical disease signs of RP in cattle and other natural hosts are similar, but show a wide variation in intensity and frequency, depending upon the virulence of the strain of virus involved, and innate resistance of the animal, breed, and species. The classic acute signs and lesions described in the literature represent descriptions from several highly susceptible breeds.

The acute disease form is characterized by a phase which lasts about 3 days with sudden onset of a febrile response (about 105-107°F) on the second or third day. The pyrexia is accompanied by depression, some loss of appetite and decrease in milk from dairy cows. The visible mucosa becomes congested and the muzzle is dry. There is clear lacrimation and a serous discharge flows from the nostrils. These discharges become more profuse later and mucopurulent as the disease progresses. There is acceleration of the respiration, heart beat and suppression of the appetite. The majority of animals begin to show mucosal lesions between the second and fifth day following onset of pyrexia. No specific clinical or differential diagnostic signs are visible during this phase.

About the fifth day of pyrexia, oral lesions appear and consist of small necrotic foci which coalesce to form more or less extensive and loosely adherent deposits. These are found on the gums, lips, cheek papillae, the bare and ventral lateral sides of the tongue and pharynx. Consequently, excessive salivation is often observed. During this time the animal loses weight, the coat becomes rough and the symptoms are aggravated. Respiration becomes painful and labored. Pneumonic complications are rare. Diarrhea usually appears on the fourth to seventh days of pyrexia. At first, it is watery but later includes fresh or altered blood. The animal becomes dehydrated, weak, prostrated, is comatose and dies. Death usually occurs on the sixth to the twelfth day after pyrexia. In sysceptible cattle herds, the mortality rate may reach 90%. In cattle with innate resistance or in enzootic areas the disease produces low mortality and less characteristic signs and lesions.

Recovery, if it occurs, will proceed dramatically about the third to fifth day after appearance of oral lesions. However, diarrhea may persist for a longer time. Recovery is complete in 4-5 weeks.

Supra infection.--Latent protozoal infections are common complications of natural RP infection and vaccination with attenuated live virus vaccines. These serious sequelae are usually sudden and commonly occur on the fourth to sixth day of illness. Frequently the clinical signs of the activated or supra infections alter the clinical disease signs and complicate diagnosis.

Diagnosis:

Presumptively, when the disease is enzootic, RP is usually diagnosed on the clinico-pathological gounds, herd history, and presence or absence of diseases which share manifestations common with RP such as virus diarrhea/mucosal diseases, papular stomatitis, infectious bovine rhinotracheitis, foot-and-mouth disease and malignant catarrhal fever. It is therefore mandatory that the causal agent be identified. The laboratory confirmation of diagnosis of RP depends upon the following:

1. Isolation of the virus from sick or dead animals
2. Detection of virus-specific antigen by the CR, AGDP and FA tests
3. Demonstration of the development of antibodies
4. Histological examination of virus-specific changes

Virus isolation is carried out by inoculation of infected blood samples and lymphoid tissues in susceptible and immune animals. The virus isolate must be identified by inhibition of its infectivity with immune serums.

Virus recovery by inoculation of animals or tissue culture always requires a longer time then could reasonably be allowed in the confirmation of outbreaks in a virgin epizootic. The detection of virus-specific antigen whether by CF or by AGDP is relatively quicker than by the virus isolation method. The agar gel diffusion test is less sensitive than complement fixation and the disadvantage is that both antigens are heat sensitive.[45] Both tests are ineffective very early in the course of the disease and also later after appearance of antibody. Fortunately, infectious virus can be demonstrated at these times. Material for use in both tests can be obtained by lymph node biopsy.

Regardless of the method used for diagnosis, successful isolation of RPV is dependent upon proper sampling and the optimal time of collection. Samples of choice are those collected during the first 4 days of the fever before the onset of diarrhea. Dehydrated and emaciated animal have low titers of virus. Samples from dead animals are not satisfactory.

The successful detection of antigens, like virus isolation, depends upon satisfactory sampling. The tissues of choice are the lymph nodes and the optimal time for collection of samples is the third or sixth day of fever. Samples are taken after diarrhea are not satisfactory. Similarly many samples from dead animals are negative. Complement fixation tests for the detection of antibodies are not recommended.[45] The neutralization test conducted in cattle and rabbits is expensive and slow for use in enzootic areas. The preferred test is the quantitative antibody neutralization test[36] in which serial dilutions of serum are mixed with a constant

amount of cell culture adapted virus, incubated in the cold overnight and seeded into suspended cell cultures.

Differential diagnosis:

Supra infection of latent pathogens complicates clinical diagnosis of RP. In addition, the following diseases have clinical features common with RP: viral diarrhea, infectious bovine rhinotracheitis, acute gastro-enteric conditions such as arsenic poisoning and coccidiosis. Also, doubts at autopsy have been caused by salmonellosis, bovine petechial fever, hemorrhagic septicemia, radiation poisoning, theileriasis, and trypanosomiases. In small ruminants, differentiation from RP must be onsidered from bluetongue, Nairobi sheep disease and sheep pox. (See Table 1.)

Vaccination:

Many efforts were made to develop safe and economic vaccines to immunize against RP including the use of simultaneous administration of virulent RPV and bovine hyper-immune serum. The successful response of certain breeds of cattle to these vaccines and the adverse effect of the same vaccines in other breeds or species of the same breed led to evolution in the adaptation of the virus in several hosts. Rinderpest was first adapted in goats and then in rabbits. This was followed by adaptation of the lapinized virus in chicken embryos. More recently, the virulent Kabete "O" strain of RPV was passaged and adapted in monolayers of calf kidney cells.

The stable characteristics of cell culture vaccines with its high degree of attenuation for cattle[35] and its inability to spread by

contact makes it the vaccine of choice for RP.

Cattle injected with cell culture vaccine resisted challenge with virulent virus 14 days later.[15,35] Protection through interference developed within 3-5 days of vaccination.[15,35]

The tissue culture vaccine was the vaccine which was used in the joint RP vaccination campaign in Africa beginning in 1961.

Inactivated vaccines:

Inactivated vaccines are advantageous in their safety. They do not evoke systemic reactions and do not activate latent pathogens. However, they are not suited for mass vaccination because of their cost and bulk. They also induce a short-term immunity. The range of chemicals employed to inactivate the virus were glycol, phenol, tulol, formalin and chloroform. Beta-propiolactone (BPL) also inactivated the virus, but as yet has not been used in production of vaccine.

Control:

Rinderpest has been eradicated from many parts of the world, but enzootic foci still exist. In enzootic areas adults are immune either through recovery from natural attacks of the disease or from vaccination. The young are protected passively through ingestion of antibodies in the colostrum. The yearlings, however, are susceptible but because of their higher innate resistance, the disease is mild or inapparent and the mortality is low. Imported stock often suffer most.

Control of virgin outbreaks in disease-free countries is accomlished by the slaughter of infected and contact ruminants and swine together with application of strict quarantine measures and movement

of cattle. High risk countries with surrounding infected neighboring areas seal their borders to create an immune barrier zone. Cattle in the barrier zones are vaccinated and restriction is imposed on movement of animals.

Vaccination is practiced regularly in enzootic areas; however, successful control of rinderpest in a certain country is achieved by vaccination of all involved livestock and also those in neighboring countries.

TABLE 1

COMPARISON OF RINDERPEST WITH OTHER DISEASE CONDITIONS

	Rinderpest Virus (RNA)	Arsenic Poisoning	Malignant Catarrhal Fever (Herpes)	Bovine Viral Diarrhea Virus (Toga)
Epithelial erosions	++++	-	++++	++++
Occular lesions	±	-	++++	±
Incubation - Experimental/days	3-9	sudden	15-63	2-3
Disease Course/days	8-15	-	10-30	7-21
Mortality	high	high	low	low
Morbidity	high	low	high	high
Subclinical	low	-	high	high
Diarrhea	++++	+++	++	++++
Peyer's patches hemorrhage	++++	-	++	++++
Gastrointestinal hemorrhage	++++	+++	+++	++++
Temperature (acute)	high	-	high	high

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SHEEP POX*

I. Identification of disease:

A. Definition:--Sheep pox (variola ovina, Clavelée-Pockenseuche) is a highly contagious viral disease of sheep characterized by erythematous eruptions on the skin. Early in the course of the disease, sheep pox lesions are papular but later progress to pustular eruptions. When the lesions are generalized, they may be associated with hemorrhagic inflamation of the respiratory and gastrointestinal mucosae and high mortality.

B. Etiology.--The viral entity of sheep pox and its characteristic inclusion bodies were established in 1902. The size of sheep pox virus is 200-250 x 150-200 mu. It has been propagated in a variety of cell cultures, but it replicates best in lamb kidney and testicle cultures, producing cytopathogenic effects and cytoplasmic inclusion bodies. Passage of the virus in cell cultures or chicken embryos results in attentuation for sheep. Although all virus strains of sheep pox are serologically identical, strains may vary in virulence.

Under natural conditions sheep and goat pox viruses are host specific. However, their immunogenic relationship was confirmed by reciprocal cross protection, complement fixation (CF) and agar gel diffusion precipitin (AGDP) tests.

Sheep pox virus is related antigenically to Neethling lumpy skin disease virus. Although cross protection was not demonstrated between sheep pox and contagious pustular dermatitis, its serological relationship was shown by the CF and AGDP tests.

Sheep pox virus in lymph retains its infectivity for 2-3 years at -15°C and for 2-3 weeks at 25-37°C. It is inactivated when heated

*Prepared by A.H. Dardiri

3 hours at 50°C, 15 minutes at 52-53°C and 3 minutes at 56-58°C. Virus produced in cell culture is inactivated by ether and chloroform after 18-24 hours exposure. Sheep pox virus in lymph is inactivated in 5 minutes by 2% phenol, 0.5-1% formalin, 0.1-1% hypochloride, 2% hydrochloric acid, sulphuric acid and 1:10,000 tincture of iodine.

C. History.--Sheep pox has been described in Europe since the first century A.D. It was reported in England, France, Italy and Germany during the 13th century. Severe epizootics were encountered in the countries of Southeastern Europe and the Mediterranean regions, approximately 1,000,000 sheep succumbing to the disease in 1805. Through the use of vaccine and application of sanitary measures, the disease was controlled in Britain, Germany and other Western European countries before the end of the 19th century. Foci of infection still exist in France, Spain and Portugal.

II. Signs:

A. Clinical Features.--The initial disease signs are fever, lacrimation, salivation and serous nasal discharge. Approximately 2 days later eruptions develop in the sparsely wooled areas of the skin such as the groin, scrotum, area below the tail, eyelids, lips, cheeks, nostrils, udder and vulvar labia. Sheep pox lesions begin as macules with a slight edema of the surrounding skin. Later the lesions develop into papules followed by pustules. The formation of pustules may be preceded by the development of vesicles; however, development of vesicles is not always observed. As the surfaces of the pustules dry out, thin scabs are formed. The benign form of the disease is more common in adult animals with skin lesions, particularly under the tail, and a mild systemic reaction and mortality of about 5-10%. A malignant form of sheep pox is more common in lambs, with depression, generalized and coalescent skin

lesions and frequently other lesions in the buccal, digestive and respiratory mucosae. Secondary bacterial infection may elicit a second temperature rise. Mortality can reach 80% of the effected flock in this severe form.

Susceptibility to sheep pox varies with age, breed, resistance of individual animals, and husbandry of the flock. The course of the disease is usually 3-4 weeks. Certain breeds of sheep are resistant to natural and experimental infection. Marino sheep are highly susceptible.

B. Incubation period.--The incubation period of sheep pox varies from 2-14 days. Usually it is shorter during hot weather.

III. Pathologic changes:

A. Postmortem lesions.--Skin eruptions or pocks on the areas of the skin devoid of wool are common. The cutaneous areas surrounding these lesions are hyperemic with edema of varying degree. All, or a combination of lesions, such as papules, vesicles, pustules, pocks and scabs may be found. Rupture of the pustules usually results in matting of the wool surrounding the pustule. Lesions in lambs are often coalescent. In the malignant form, pox lesions may extend into the mucosa of the mouth, pharynx, larynx and vagina. Small grayish lymphoma-like or caseated nodules surrounded by pneumonic areas are often found in the lungs and kidneys.

B. Microlesions.--Edema of the dermis with infiltration of polymorphoneculear cells is noted in the macular stage of sheep pox. In the papular stage, large cells resembling histiocytes are present. Later, these cells are transformed into "sheep pox cells" or "cellules claveleuses", which are large cells with oval or irregular nuclei. Some of these cells contain granular cytoplasmic inclusion bodies. Accompanying

the appearance of "pox cells", is an inflammatory edema which may extend to the subcutis. Necrosis and thrombosis of the dermal veins are often present. Scab portions of the lesion consist of exudate and a pellicle of necrotic epidermis.

IV. Diagnosis:

A. In the field.--Appearance of a progressive pox-like disease in a susceptible sheep flock is suggestive of sheep pox, especially when asssociated with movement of animals or introduction of new stock. However, clinical diagnosis may be difficult in the mild form where lesions are confined to small areas of the skin.

B. Laboratory.--Direct light microscope examination of stained smears from fresh lesions will assist in the diagnosis of sheep pox by revealing typical inclusion bodies. Electron microscope examination of lesions material will rapidly demonstrate morphology of the virus particle. Identification of sheep pox antigen in infected cells by staining with fluorescein or ferritin conjugated antibodies is necessary for a confirmatory diagnosis. The virus may be isolated from the blood, lymph and lesions of affected animals during the viremic stage and by inoculation of cell culture, chicken embryos or susceptible sheep. Detection of specific antibodies in the serum of recovered animals can be done by virus neutralization, CF, AGDP and fluorescent antibody (FA) tests. Complement fixation antibodies are detected in the serums of vaccinated or artifically infected sheep as early as the 7th day after inoculation and reach a peak at 21-26 days postinoculation (DPI). Precipitating antibodies are detected as early as 14 DPI. The following specimens may be submitted to the laboratory frozen with dry ice: 1) blood from sheep during the febrile disease stage, 2) lymph node and scab lesions, and 3) serum obtained at the acute and

convalescent stages of the disease. A portion from skin lesions should be prepared in buffered glycerin.

C. Differential Diagnosis.--Formation of scab-like lesions are common to sheep pox, eczema and scabies. Eczema is non-infectious whereas the last is a parasitic disease. In their non-complicated form none of them are associated with a febrile reaction. The mouth lesions and the systemic reaction of sheep pox may be confused with those of peste des petits ruminants (PPR). Lack of papule and pustule formations on the skin and presence of necrotic ulcerative stomatitis in animals infected with PPR will assist in the differential diagnosis of the two diseases. Sheep pox may also be confused with contagious ecthyma (CE); however, proliferative lesions of the disease around the mouth in and

V. Prognosis:

Mortaility from sheep pox varies from 5-80%. Losses from the disease may be increased by the stress factors of severe hot weather and unsatisfactory snaitary husbandry practices. Outbreaks of sheep pox are more common during the summer. Lesions as well as mortality are more severe in lambs than in older sheep. The peak of mortality is usually within 2 weeks following appearance of generalized and coalescent lesions. Severity of the disease varies with each epizootic and the particular breed of sheep involved.

VI. Epizootiology:

A. Geographical Distribution.--Sheep pox in various areas of Europe, Asia and Africa and is enzootic in Iran, India and neighboring countries. Foci of the disease are found in Spain, Portugal and Russia. It has been reported in Egypt, the Sudan, Ethiopia and Kenya. It has not

been recognized in North and South America.

B. Transmission.--Outbreaks originate by introduction of or contact with infected sheep. Sheep pox is spread by aerosols from nasal secretions, saliva and dried scabs. The disease is transmitted by direct contact of susceptible and sick animals and indirectly by contaminated fomites and transport vehicles. Experimental disease transmission is possible by intradermal, subcutaneous, intravenous, intraperitoneal and intracerebral inoculation. Administration of the virus into the nasal cavity or trachea provokes generalized disease in susceptible sheep. Sheep pox virus may remain viable in wool for 2 months and on contaminated premises for as long as 6 months.

C. Hosts.--Sheep are the natural hosts for sheep pox virus. The virus is host-specific although infection of other species can occur experimentally. Artificial exposure to sheep pox protects cattle against lumpy skin disease. Some strains of sheep pox virus can be adapted to goats with loss of virulence for sheep.

VII. Control and Eradication:

A. Preventive Measures.--The introduction of sheep from countries where sheep pos is enzootic to those countries free of the disease should be prohibited.

B. Control and Eradication.--In the United States of America the disease must be reported to the State and Federal regulatory agencies. Imposition of quarantines on infected premises is mandatory. Movement of sick and contact sheep should be prohibited. Laboratory diagnosis should be obtained as soon as possible. Infected and contact sheep flocks should be slaughtered and the carcasses disposed of properly. Infected premises should be thoroughly cleaned and decontaminated.

C. Immunization.--Two types of live, virulent vaccines were commonly used until the pox virus was attenuated by passage in both chicken embryo and lamb kidney cell culture. Mild virus strains which did not cause generalized lesions following subcutaneous or intradermal inoculations were used in Iran and Egypt. Later in Russia an attenuated strain of sheep pox virus was obtained by passage through chicken embryo cultures. Its safety and efficiency as an immunizing agent was proven by extensive field trials. More recently, sheep pox virus was attenuated after 30 serial passages in sheep kidney cell cultures and used in Iran. The duration of immunity obtained with this vaccine under laboratory and field conditions was at least 22 months, combination of this vaccine with one against anthrax administered as a single injection resulted in a solid immunity against both diseases. Trials conducted in India to develop chemically inactivated vaccines. Crude virulent virus was inactivated after 48 hours with 0.01% formalin. This product adsorbed to aluminum hydroxide gel, protected sheep for at least 4 months.

VIII. Public Health:

Man is not susceptible to sheep pox under natural conditions. Sheep slaughtered during the febrile of pyemic stages of sheep pox are not suitable for human consumption.

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SWINE VESICULAR DISEASE *

I. Identification of disease:

A. Definition.--Swine vesicular disease (SVD) is a contagious, viral infection of swine. It is characterized by vesicular lesions and subsequently by erosions of the epithelium of the mouth, nares, snout, and feet.

B. Other vesicular diseases.-- The clinical lesions and signs of pigs suffering from SVD are indistinguishable from those due to foot-and-mouth disease (FMD), vesicular stomatitis (VS), and vesicular exanthema of swine (VES).

C. Etiology.--Swine vesicular disease is caused by an enterovirus of the picornaviruses (1). It is not affected by acid, pH or ether, contains single-stranded RNA and is of 280 Å diameter. Its density in cesium chloride is 1.34 grams per ml and it has a sedimentation rate of 150 S. It is stabilized by 1M $MgCl_2$ at 50°C.

The virus of SVD is closely related to Coxsackie B-5 which is a human enterovirus (5). All strains of SVDV are neutralized by anti-Coxsackie B-5 virus antiserums and serum from pigs recovered from SVD will neutralize Coxsackie B-5 virus in tests in tissue cultures of mice. Antiserums to other pig enteroviruses show no neutralizing activity against SVDV. Pig serums collected in the field from animals in the U.S. did not neutralize SVDV whereas several human serums did, indicating that an infection had occurred in these persons by a virus related to SVDV.

Strain variation among different isolates of SVDV can be readily demonstrated by double diffusion reactions in agar (6). By use of this

*Prepared by J.J. Callis, J.H. Graves and P.D. McKercher

procedure it was shown that illness that had occurred in some laboratory workers was due to infection by SVDV and not Coxsackie B-5 virus. Hybridization studies of the RNAs from different SVDV strains and Coxsackie B-5 show about 50% homology between SVDV and the Faulkner strain of Coxsackie B-5 (Brown, personal communication). However, it also has been shown that Coxsackie B-5 virus recently isolated from human cases shown about the same degree of identity with the B-5 strains isolated 20 years ago, indicating a variation of the virus has occurred through the years.

Based on the absence of evidence of any previous infection of pigs with a virus serologically related to SVDV and its close relationship to the human virus, Coxsackie B-5, it has been suggested that SVD is a relatively new disease of pigs caused by a virus of human origin (5).

D. History:-- Swine vesicular disease was first recognized as a distinct entity of swine in Italy in 1966, where Nardelli et al. (1) reported on a syndrome in pigs that resembled foot-and-mouth disease (FMD). The disease was milder but the lesions were indistinguishable not only from those of FMD, but vesicular exanthema of swine (VES) and vesicular stomatitis (VS). Nardelli, together with British colleagues, indicated that the agent responsible for this outbreak was a porcine enterovirus. Following the initial outbreak the disease subsided and the peculiar incident was soon forgotten.

In 1970, Mowat et al. (2) from the Animal Virus Research Institute at Pirbright, England, noticed a vesicular condition in pigs in an area of Hong Kong where they were conducting trials with experimental FMD vaccines. The disease differed from FMD only in that the morbidity was lower, lesions less severe and there was no mortality. If first appeared in pigs on a farm where the swine had not been vaccinated with the experimental vaccine.

It subsequently spread to neighboring farms and others up to seven miles away. Repeated tests failed to show the virus to be any of the seven serotypes of FMDV. Bearing in mind that one of the seven recognized types of FMDV was first identified in material sent from Asia, the British workers were aware that this outbreak might be due to a new serotype. The possibility that a new serotype may evolve in the field must always be kept in mind, and especially with virus from this area where movement of animals is frequent with China to the north. The problem was simplified by the previously recorded Italian outbreak and the availability of diagnostic materials. The results of the tests indicated a close similarity between the viruses from the Hong Kong and Italian outbreaks which led to Hong Kong virus being classified as a porcine enterovirus.

In December of 1972 (3), a vesicular disease occurred among pigs on a farm in England and based on clinical signs was confirmed as FMD. Five days later, however, when repeated serological tests failed to identify FMD antigen, the identification of the agent as an enterovirus was confirmed. By comparison with the Hong Kong and Italian viruses, it was concluded that the vesicular disease occurring in England was due to a virus similar to that which had been identified in Italy and Hong Kong. It was evident that a new disease entity was present in England. The eradication procedures that had been already instituted were modified in that only swine were slaughtered.

The European Commission for the Control of Foot-and-Mouth Disease, FAO convened a meeting in Rome in January of 1973, to assess the situation in Europe and to adopt standard diagnostic procedures (4). At this meeting it was disclosed that SVD had continued to occur periodically in

Italy since 1966, but that the lesions were milder than those originally observed. Further, the delegates from Poland and Austria advised that SVD had also occurred recently in their countries. To date, the disease in Europe has also been diagnosed in France, Switzerland, West Germany, and Belgium, and in Asia, in Taiwan and Japan. Efforts to eradicate the disease in England have continued and according to the latest reports it has occurred on a total of 259 premises and the British Government has spent 11 million dollars in an effort to eradicate it from that country. The disease status in at least some of the other countries is not known and it may be endemic in some. In some countries, for example Belgium and Japan, it has been eradicated by slaughter of infected and exposed animals and decontamination of the premises.

Swine vesicular disease is of great economic importance. It causes vesicles on the feet, snout and in the mouth resulting in lameness and difficulty in eating. The animal loses weight and becomes unthrifty. The economic importance of the disease to a country that exports swine or pork products is greater because countries free from the infection usually close their markets to products from the affected country. The direct costs of eradication may also be considerable.

II. Signs:

A. Clinical features.--The clinical signs and lesions of SVD are indistinguishable from those of FMD, VS, and VES (see foot-and-mouth disease). The first evidence of infection is lameness which often precedes the appearance of vesicles by several hours. Diseased pigs have fever of 104 to 106 F or greater. Feed comsumption is reduced, probably due to reluctance to move to the feed troughs.

The lesions are vesicular and appear on the coronary bands, soles of the feet, interdigital spaces and tongue, nares, and lips. Ulceration of the skin over the metacarpal and metatarsal regions is common. While not documented, abortion or death of newborn pigs has been observed. The vesicles rupture and often epithelial areas of feet not originally vesicular will lift away and form raw ulcerous areas. Occasionally, the hoof will be shed from the infected foot.

There is increasing evidence that a subclinical form of SVD occurs or a mild form that goes undetected unless careful examination is made. This has complicated eradication campaigns in that the disease can be widely disseminated before the diagnosis is made.

Preliminary studies of SVD at PIADC indicated that infected pigs developed encephalomyelitis (9). In Italy, Gagliardi et al. (10) have seen encephalitis as a clinical feature of the disease in at least three different breeding establishments. Some signs of central nervous system involvement consisted of a very unsteady gait resulting in short stiff-legged steps as if unsure of their equilibrium. Shivering occurred across the flanks and chorea type leg movements were frequently observed (Graves et al., unpublished data). In one experiment, a high mortality rate was found in newborn pigs. In this experiment a sow was infected by intravenous inoculation of SVDV-UKG within 15 hours after delivering 9 normal pigs. All of the piglets were dead by the third day after the appearance of clinical signs in the sow (McKercher et al., unpublished data).

Swine apparently are the only species of livestock susceptible to SVD.

B. Incubation period.--Susceptible animals in contact with SVD-infected pigs will show signs of the disease in 2 to 7 days. If the virus is inoculated, signs may be seen as soon as 30 hours after inoculation. Pigs fed contaminated feed showed evidence of SVD in 2 to 3 days.

III. Pathologic Changes:

A. Pathogenesis.-- The primary sites of infection in SVD appear to be the intestinal tract. Infection is readily obtained by feeding the virus. The disease can be induced by inoculating the virus on the feet or intravenously. The sensitivity of other routes of infection has not been extensively studied.

Virus is widely disseminated in the infected animal being found in essentially all tissues. It is readily isolated from the feces, blood, and OP samples of diseased pigs.

Neutralizing antibody against SVDV can be found in the serum of infected pigs by the 7th day postinfection rising to a peak by the 28th day (11). Neutralizing antibody persists in recovered pigs for at least 60 days after infection at which time the recovered swine are refractive to infections (McKercher and Graves, unpublished date). An experimental vaccine was prepared by inactivating SVDV grown in IBRS-2 cells for 72 hours with 0.05% acetylethyleneimine at 25°C and combining the inactivated antigen with an oil adjuvant. Vaccinated pigs were refractive to challenge by inoculation or exposure to infected animals at 60 days postvaccination (McKercher, 1973, unpublished date).

B. Post-mortem lesions,--Extensive study of the pathology of SVD-infected pigs has not been reported. Evidence of damage to the feet

from vesiculation may be seen in pigs up to 30 days after recovery. Monlux et al. (1974) found extensive microscopic lesions including perivascular cuffing and infiltration by polymorphonuclear leucocytes, other lesions were related to vesiculation and similar to FMD.

IV. Diagnosis:

A. Field diagnosis.--Any vesicular disease in pigs is of major importance. It is impossible to distinguish SVD from the other vesicular diseases of swine in the field, but information on the possible involvement of cattle in the area may be an important clue. A word of caution, however, is that occasionally a strain of FMD may be present that does not readily infect cattle.[1] (See the section on foot-and-mouth disease for differential diagnosis of vesicular diseases.)

B. Field samples.-- See chapter on FMD and section on collection of samples for vesicular diseases.

C. Laboratory diagnostic tests.--(See chapter on FMD). Differentiation from FMD, VES and VS can only be done by use of laboratory serological procedures. Of these the complement-fixation (CF) test is more rapid than the serum neutralization test.

Antiserums for use in the CF test are prepared by immunizing guinea pigs with SVDV antigen purified from tissue cultures or virus recovered from the brains of infected baby mice. The test antigen used for the differential diagnosis usually consists of a suspension of vesicular lesion material collected from the diseased animal.

Differential diagnosis by serum neutralization can be done by mixture of the test virus with the guinea pig serums used in the CF test and then

inoculating this mixture into tissue culture cells susceptible to virus infection.

Differences among strains of SVDV can be demonstrated by observation of "spurs" that occur where heterologous and homologous antiserums are used in double diffusion precipitin reactions in agar (6). Such differences are of importance in epizootiological studies of SVD and are not of concern in the differential diagnosis within the vesicular disease group.

Some differentiation can be shown by inoculating the test virus into different lines of tissue culture cells (IBRS, PK 15). The SVDV had only been shown to grow in cells of pig origin whereas FMDV will grow in a variety of bovine cells and BHK cell lines.

Newborn mice less than 24 hours old are susceptible to SVDV and will die within 3 to 7 days post intra-cranial inoculation. Mice become resistant when older than 6 days.

V. Prognosis:

Prognosis of recovery from SVD is generally favorable; however, the existence of this disease as an endemic problem will severely hinder the diagnosis and ready detection of the presence of FMD.

VI. Epidemiology:

A. Geographical distribution.--Currently (1975), SVD has been confirmed as occurring in Italy, Poland, Austria, France, Great Britain, Hong Kong and Japan.

B. Transmission.--The primary mode of transmission is by contact of susceptible pigs with the excretions of infected pigs. The virus is much more resistant to disinfectants and environmental conditions than

FMD virus and during the attempted eradication of the disease from Great Britain in 1973, infection of susceptible animals used to restock decontaminated premises proved a problem. During this same campaign it was found that trucks that had carried infected pigs and had been decontaminated by standard FMD procedures were a major source of subsequent spread of the disease.

Investigations of outbreaks of SVD seem to incriminate the feeding of garbage contaminated with SVDV-infected meat scraps. The stability of the virus is such that it is not inactivated by the acid changes that occur in the musculature after death; thus, the virus can be expected to withstand the various processes used in the production of sausages such as salami. Dried salami and pepperoni sausages prepared from the meat of infected swine were found to contain residual SVDV for at least 200 days. Processed intestinal casings from infected swine, packed in salt and stored at 39 F, contained virulent virus for a similar period. Thus, imported pork products produced in countries where SVD is present are potential sources of infection to a susceptible swine population (16, 17).

Those cured and dried products originating from countries where SVD is present are not permitted entry into the United States except for further processing by heating to an internal temperature of 166 F.

C. Hosts:--The only known species susceptible to infection with SVD are swine, baby mice, and man. (See Table 1).

VII. Control and Eradication:

A. Prevention.--(See foot-and-mouth disease.)

B. Control and eradication.--(See foot-and-mouth disease.)

C. Treatment.--(See foot-and-mouth disease.)

D. Immunization.--An experimental vaccine against SVD has been reported (12, 13, 14). Although vaccines have not been used other than experimentally, the data provided would indicate that they are quite adequate.

E. Import restrictions.--(See foot-and-mouth disease.)

VIII. Public Health Aspects:

Swine vesicular disease virus is closely related to the human enterovirus, Coxsackie B-5. Human infection has been reported in laboratory workers and most human serums will show some neutralization of SVDV. Caution should be taken in handling highly virus-contaminated materials and unnecessary human contact with diseased pigs should be avoided.

TABLE 1.

SUSCEPTIBILITY RANGE TO VIRUSES OF FOOT-AND-MOUTH DISEASE (FMD), VESICULAR EXANTHEMA OF SWINE (VES), VESICULAR STOMATITIS (VS), AND SWINE VESICULAR DISEASE (SVD)

Species	Virus			
	FMD	VES	VS	SVD
Horse	0	±	+	0
Bovine	+	0	+	0
Swine	+	+	+	+
Sheep & Goats	+	0	+	0
Man	± (Rare)	0	+	±
Mice, 1-2 days	+	0	+	+
Adult Mice	±	0	+	0

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VESICULAR EXANTHEMA OF SWINE *

I. Identification of disease:

A. Definition.--Vesicular exanthema of swine (VES) is an acute, febrile, contagious viral disease of swine, characterized by formation of vesicles on certain parts of the body.

B. Etiology.--The causal agent of VES is a virus of the picornovirus group, and it is different from the other three major vesicular diseases, foot-and-mouth disease (FMD), vesicular stomatitis (VS), and swine vesicular disease (SVD).

C. History.--On April 22, 1932, a vesicular disease affecting swine only was first reported from a hog ranch near Buena Park, Orange County, California. The 1932 outbreak was diagnosed FMD and animals involved were slaughtered. In 1933 a similar disease occured in San Diego, about 100 miles distance from the 1932 focus. Following this outbreak, the malady was correctly described as a new disease. Sporadic outbreaks occurred in the USA until 1956, the last taking place in New Jersey that year.

II. Signs:

A. Clinical features.--A characteristic temperature rise is usually followed by vesicles of the snout, lips, or oral cavity as well as the coronary band and interdigital regions of the feet.

B. Incubation period.--Primary lesions develop within 12-48 hours and secondary lesions 12-48 hours later.

III. Pathological changes:

A. Post mortem.--Vesicles and similar lesions affecting the dermis and contiguous mucosae on the affected parts constitute the usual lesions.

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*Prepared by R. J. Yedloutschnig and F.W. Wilder

B. Microlesions.--Virus replicates in the Malpighian layer of the epidermis; concurrently, the stratified squamous epithelial cells undergo marked swelling of their cytoplasms. Following this the cells become necrotic; their deterioration spreads virus to non-infected cells continuing the formation of lesions.

IV. Diagnosis:

A. In the field.--Pyrexia, vesiculation (and related lesions), and lameness are always present. It should be remembered that actual vesicles may rarely be seen as they are frequently ruptured. The vesicles and lesions are not distinguishable from those of other vesicular diseases.

B. Laboratory.--Laboratories utilize various tests, including complement fixation, virus neutralization, isolation, and identification of the virus. Animal inoculation is frequently employed in the laboratory and sometimes in the field.

C. Differential diagnosis.--Final confirmation of the diagnosis can only be made by the laboratory tests mentioned above. However, the inoculation of equine, bovine, and porcine species brought from an adequate distance from the outbreak, may be used to reach a presumptive diagnosis. Equine, bovine, and porcine species are affected by VS; bovine and porcine are both affected by most strains of FMDV; VES affects only swine. The USA is now free of VES; however, since VES has been regarded as an "extinct" disease, any new vesicular outbreak affecting only swine would require laboratory determination as to whether it was a recrudescence of VES or a first outbreak of SVD.

V. Prognosis:

A severe weight loss may occur during the course of the disease but recovered of uncomplicated cases is usually prompt and without sequelae.

Fatal secondary infections may occur.

VI. Epizootiology:

A. Geographic distribution.--The disease has only been diagnosed in continental USA, except for a single appearance in Iceland in 1955 which was caused by feeding local swine garbage containing porcine scraps from the USA.

B. Transmission.--The disease is spread by direct contact and by feeding raw garbage. The link between raw garbage and the disease is apparently the retention of infective virus in pork scraps. Recent findings indicate that the Sea Lion may have played a part in transmission.

C. Hosts.--The only known host is swine. The role of the Sea Lion is being studied; a virus similar to VESV recovered from Sea Lions has produced experimental vesicular lesions in swine.

VII. Control and eradication:

A. Preventive measures.--Slaughter and quarantine of infected and exposed swine and decontamination of premises are the most effective measures. There must be absolutely no feeding of raw or improperly cooked garbage to swine.

B. Sanitation and disinfection.--A solution of 2% sodium hydroxide is a practical disinfectant. A viricide having a high pH is required to denature VES viral protein.

C. Treatment.--None.

D. Immunization.--There is no vaccine available. At the present time there are about a dozen distinct antigenic types of VESV known. Since there have been no outbreaks since 1956, research on this subject has been extremely limited.

VIII. Public health aspects:

The virus does not infect man.

Selected References:

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O U T L I N E G U I D E

Collection and Submission of Specimens

in Suspected Foreign Animal Diseases*

For Demonstrations and Exercises

See Part IIIB (DIAGNOSIS OF SUSPECTED EMERGENCY ANIMAL DISEASES) in "Emergency Animal Disease Eradication Guide".

*Prepared by F.W. Wilder, D.H. Ferris, J. Kopec and A.H. Dardiri

Introduction

The capability of a laboratory to confirm the diagnosis of a suspected exotic animal disease is directly related to the types, amounts, and conditions of the specimens submitted. Therefore, it is important that each field diagnostician become intimately familiar with this subject.

This outline is organized in approximately the same order as you would perform your specimen collection in the field. Keep in mind that specimens for agent isolation should be collected as aseptically as possible; these should always be collected prior to those for histopathology which need only be clear and fixed in 10% neutral buffered formalin.

Only diseases demonstrated in the PIADC foreign animal disease courses are included in this outline. The technics outlined are very similar, but in less detail, than those given in the well-known "Red Book" (Emergency Disease Eradication Guide). The entire part III B of this book, covering the collection and submission of specimens is included as Appendix 2, for your convenience. It should be remembered that the outlines have been developed to emphasize the principles of proper collection of laboratory specimens. Some variations in technic are included. For example, some laboratory personnel prefer tissues for histopathology cut 3 mm instead of one-fourth inch (approximately 6 mm) in thickness and fixed in 50 X rather than 10X the specimen volume of fixative.

Specimens

Certain basics such as the following should be kept in mind:

1. Ordinarily, put each tissue in a separate container, correctly labelled.
2. Use some method of labelling which cannot be lost or destroyed easily. For instance, adhesive tape should go entirely around

the vial or bottle and overlap so that moisture does not dislodge it.

3. Writing should be with pencil or ink which will not smudge or blur when wet.
4. Vials, tubes, or bottles to be frozen should be no more than half full. These should be sturdy enough to withstand freezing and dropping.
5. A history should accompany specimens.

SUGGESTIONS FOR IMPROVEMENT OF COLLECTION AND SUBMISSION METHODS:

You are invited to turn in to the director any suggestions for improvement. (Remember, that the APHIS "Red Book" is the authority for field diagnosticians in the collection and transmission of specimens.)

AFRICAN HORSE SICKNESS

1. Blood.
   a. Whole blood, in an equal volume of oxalate, phenol (carbolic acid), and glycerin (OPG or OCG) solution. Collect at height of pyrexia. Refrigerate only. (For virus isolation.)
   b. Serum, 20 ml, from acutely ill and convalescent animals, frozen. (For serology.)
   c. Blood smears; at least 6 slides, air dried and fixed in absolute methyl alcohol. (For differential counts.)
2. Tissues, collected aseptically, frozen. (For virus isolation.)
   a. Spleen, in 50% buffered glycerin.
   b. Liver.
   c. Lung.
   d. Lymph nodes draining affected regions (thoracic, mediastinal, and mesenteric).
3. Tissues, not over ¼" thick, fixed in 10X tissue volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Spleen.
   b. Liver.
   c. Lung.
   d. Kidney.
   e. Heart.
   f. Lymph nodes.
4. Formulas. See appendix 1.
5. History to be submitted with specimens. See appendices 3 and 5.

AFRICAN SWINE FEVER (AND HOG CHOLERA)

1. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, 50 ml, frozen. (For serology.)
2. Tissues, collected aseptically, frozen. (For virus isolation and FA tests.)
   a. Spleen.
   b. Lymph nodes (cervical and visceral).
   c. Liver.
   d. Kidney.
   e. Tonsil (whole or biopsy).
   f. Ileum (terminal 3 inches). NOTE: SEE APPENDICES 2 AND 6.
3. Tissues, ¼" inch or less thick, fixed in at least 10X their volume of neutral 10% formalin.* (For histopathology.)
   a. Spleen.
   b. Tonsil.
   c. Liver.
   d. Adrenal.
   e. Kidney.
   f. Lymph nodes (body and visceral).
   g. Brain (fixed intact). NOTE: SEE APPENDIX 2.
4. Formulas. See Appendix 1.
5. History to be submitted with specimens. See appendices 3, 5, and 6.

*Some laboratory personnel recommend that tissues for histopathology be cut 3 mm thick and that all tissues for histopathology be fixed in as much as 50X the tissue volume of fixative.

DERMOPATHIC BOVINE HERPESVIRUS INFECTIONS AND LUMPY SKIN DISEASE

1. Blood.
   a. Whole blood, frozen. (For virus isolation.)
   b. Serum, 20 ml from acutely ill and convalescent animals and paired sera from an individual animal in acute and convalescent phases 92 to 3 weeks after acute stage). (For serology.)
2. Tissues, frozen. (For virus isolation.)
   a. Skin lesions. Biopsies should be taken of at least 2 lesions. Scrapings from tissues and scabs, vesicular contents. (Examine muzzle, teats, mammary skin carefully.)
   b. Lymph nodes, swollen.
3. Tissues, not over ¼" thick, fixed in 10X tissue volume of 10% neutral buffered formalin. (For histopathology.)
   a. Skin lesions (biopsies).
   b. Lymph nodes, swollen.
4. Formulas. See appendix 1.
5. History to be submitted with specimens. See appendices 3 and 5.

CONTAGIOUS BOVINE PLEUROPNEUMONIA

1. Blood.
   a. Whole blood, 20 ml, refrigerated or frozen. (For agent isolation.)
   b. Serum, 20 ml, refrigerated from acutely ill and convalescent and paired sera from individual animals in the acute stage and 2-3 weeks later.
2. Body fluids. Collect aseptically, refrigerate or freeze. (For agent isolation.)
   a. Pleural fluid, 10 ml or more.
   b. Lung lesion exudate.
3. Tissues collected aseptically, frozen or refrigerated. (For agent isolation.)
   a. Lung lesions.
   b. Lymph nodes (bronchial and peripheral, when enlarged).
   c. Spleen, when engorged.
   d. Thyroid glands.
4. Tissues, not over ¼" thick, fixed in 10X tissue volume of neutral buffered 10% formalin.
   a. Spleen.
   b. Liver.
   c. Lung.
   d. Kidney.
   e. Brain, including floor of 4th ventricle.* (See appendix 1b.)
   f. Intact eye and conjunctiva.*
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

*For use in differentiating malignant catarrhal fever.

FOWL PLAGUE (AND NEWCASTLE DISEASE)

1. Whole birds. Submit whole birds if practical; as many as 3 acutely ill and 3 dead birds are requested by the laboratory. If this is not feasible, send the following specimens from several birds:
2. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, frozen. (For serology.)
3. Tissues, collected aseptically, frozen. (For virus isolation.)
   a. Spleen.
   b. Trachea.
   c. Lung.
   d. Proventriculus.
   e. Brain.
   f. Small intestine.
   g. Liver.
   h. Kidneys.
   i. Bone marrow.
   j. Cecal tonsils.
4. Tissues, cut less than ¼" thick and fixed in 10X their volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Brain.
   b. Spleen.
   c. Trachea.
   d. Lung.
   e. Proventriculus.

f. Ventriculus (gizzard).

g. Small intestine.

h. Liver.

5. Formulas. See appendix 1.

6. History to be submitted with specimens. See appendices 4 and 5.

LUMPY SKIN DISEASE: See Dermopathic Bovine Herpesvirus Infections.

NEWCASTLE DISEASE: See Fowl Plague.

PESTE DES PETITS RUMINANTS: See Rinderpest.

FOOT-AND-MOUTH DISEASE (AND OTHER VESICULAR DISEASES INCLUDING VESICULAR STOMATITIS, VESICULAR EXANTHEMA OF SWINE, AND SWINE VESICULAR DISEASE)

1. Vesicular specimens.
   a. Vesicular fluid, if available. Collect from unruptured vesicle separately; freeze.
   b. Vesicular lesion tissue. Collect about 5 grams in phosphate buffered glycerin. (Volumetric measurement of 5 cc liquid may serve as guide.)
2. Probang specimens (ruminants only). "Probang (O-P) fluids should be submitted in all cases, even though accompanied by voluminous vesicle harvest from the same animal." Director, Emergency Programs, change in "Red Book" 15 Nov. 1972.
   a. Esophageal-pharyngeal (O-P) fluid; 10 ml in equal amount of phosphate buffered glycerine.
3. Blood.
   a. Whole blood, 10 ml collected during febrile period, frozen. (For virus isolation.)
   b. Serum, 10 ml, from animals in the acute and convalescent stages. (For serology.)
4. Fecal sample. (For swine vesicular disease.) Collect from animals with and without lesions and contact animals. Freeze. (For virus isolation.)
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

MALIGNANT CATARRHAL FEVER (AFRICAN)

1. Blood.
   a. Collected in EDTA (1 mg of EDTA per 1 ml blood) or heparin and submitted on wet ice, or refrigerated but not frozen. (For virus isolation.)
   b. Serum from onset of disease and at death or recovery. (For serology.)
2. Tissues (for virus isolation, refrigerated but not frozen.)
   a. Spleen.
   b. Lymph nodes.
   c. Adrenal.
   d. Thyroid.
3. Tissues. (For histopathology, 1/4" slices fixed in 10 volumes of 10% neutral buffered formalin.)
   a. Kidney.
   b. Spleen.
   c. Liver.
   d. Adrenal.
   e. Lymph nodes.

RINDERPEST (NOTE: SEE APPENDIX 2)

1. Blood.
   a. Whole blood, heparinized, frozen. (For virus isolation.)
   b. Serum, 20 ml, frozen from acutely ill and convalescent animals. (For serology.)
   c. Blood smears; 6 slides air dried and fixed in absolute alcohol. (For differential diagnosis.)
2. Tissues. Collected aseptically, frozen. (For virus isolation.)
   a. Spleen.
   b. Lymph nodes (mesenteric, draining affected areas).
3. Tissues, not more than ¼" thick, fixed in 10 tissue volumes of 10% neutral buffered formalin. (For histopathology.)
   a. Mouth and tongue lesions.
   b. Spleen.
   c. Liver.
   d. Lung.
   e. • Kidney.
   f. Lymph nodes (draining affected areas).
   g. Sections from intestines.
4. Fecal sample. (For differential diagnosis.)
5. Formulas. See appendix 1.
6. History to be submitted with specimens. See appendices 3 and 5.

*Some laboratory personnel recommend cutting tissues 3 mm thick and fixing in 50X.

SWINE VESICULAR DISEASE: See FMD.

VESICULAR EXANTHEMA OF SWINE: See FMD.

VESICULAR STOMATITIS: See FMD.

SHEEP POX

1. Blood.
   a. Whole blood taken during febrile period and submitted frozen.
   b. Serum from the acute and convalescent stages.
2. Lymph nodes and lesions.
   a. Submit frozen.
   b. Submit in buffered glycerin.
   c. Submit small sections frozen on metal foil for FA.

APPENDIX 1

1. OPG or OCG Oxalate Phenol Glycerine Solution.

   Potassium oxalate..... 5 gm

   Carbolic Acid (Phenol)..... 5 ml

   H_2O.....500 ml

   Glycerine.....500 ml

2. Glycerol (Glycerin) Buffer, pH 7.2 (Phosphate Buffered Glycerin).

   a. Monobasic Sodium Phosphate (NH_2PO_4,$2H_2O$)

      31.2 g per L, distilled H_2O

   b. Dibasic Sodium Phosphate (Na_2HPO_4,7 H_2O)

      53.65 g per liter of distilled H_2O

   Add 28 ml solution A = 72 ml solution B to an equal volume of glycerol.

3. Ten Percent Neutral Buffered Formalin.

   Monobasic Sodium Phosphate ($NaH_2PO_4 \cdot 2H_2O$)..... 4.0 gm

   Dibasic Sodium Phosphate (Anhydrous Na_2HPO_4)..... 6.5 gm

   Distilled H_2O ..... 900 ml

   Formalin (40%)..... 100 ml

   Use a volume of above at least 10 times the volume of tissue. (For a few tissues, some personnel recommend 50X.)

4. Disodium Ethylenediamine Tetraacetate (EDTA). Use 0.5 to 1 mg per 5 ml of blood as an anticoagulant.

5. Heparin. Use 0.1 to 0.2 mg per ml of blood as an anticoagulant.

6. Sodium Citrate Anticoagulant.

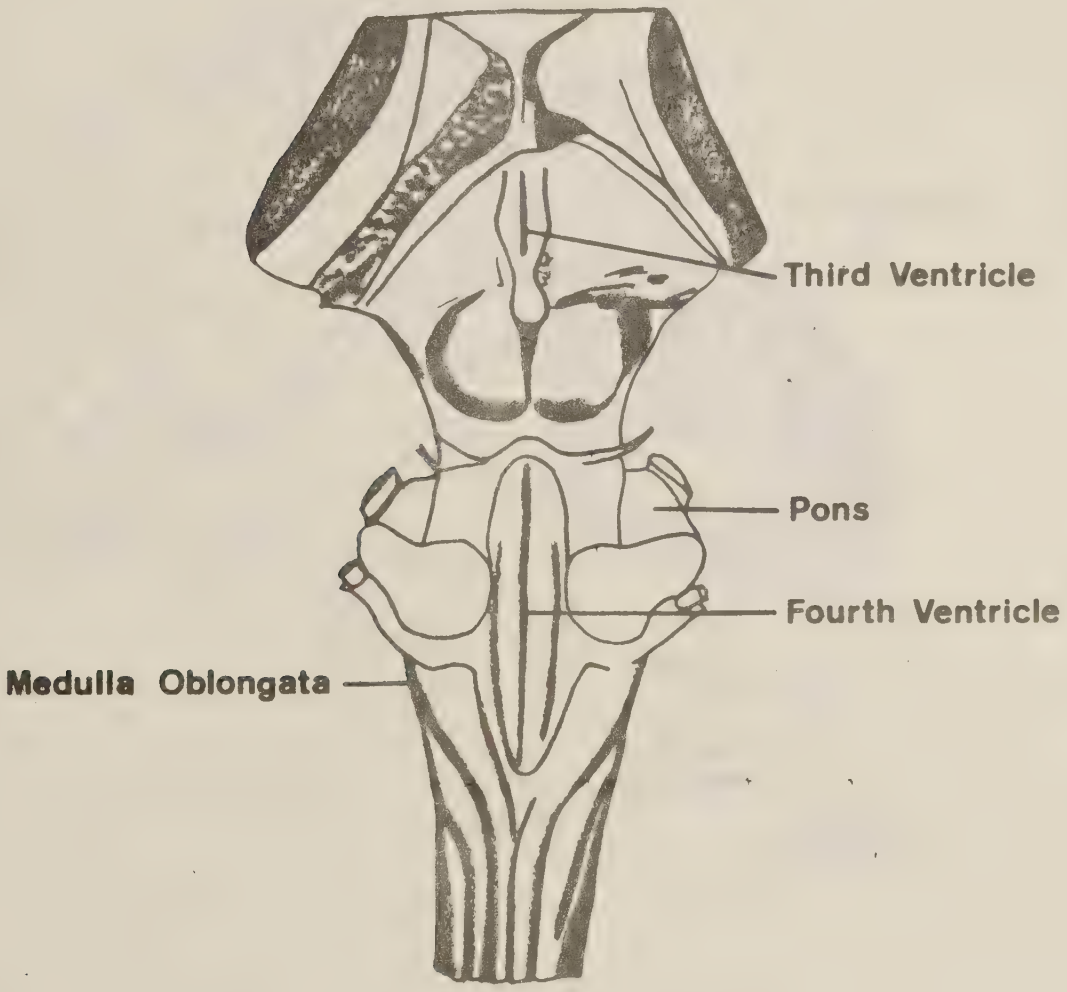
   Sodium citrate..... 10.0 gm

   H_2O (dist.)..... 100.0 ml

   Sterilize by autoclaving: 1 ml per 10 ml blood.

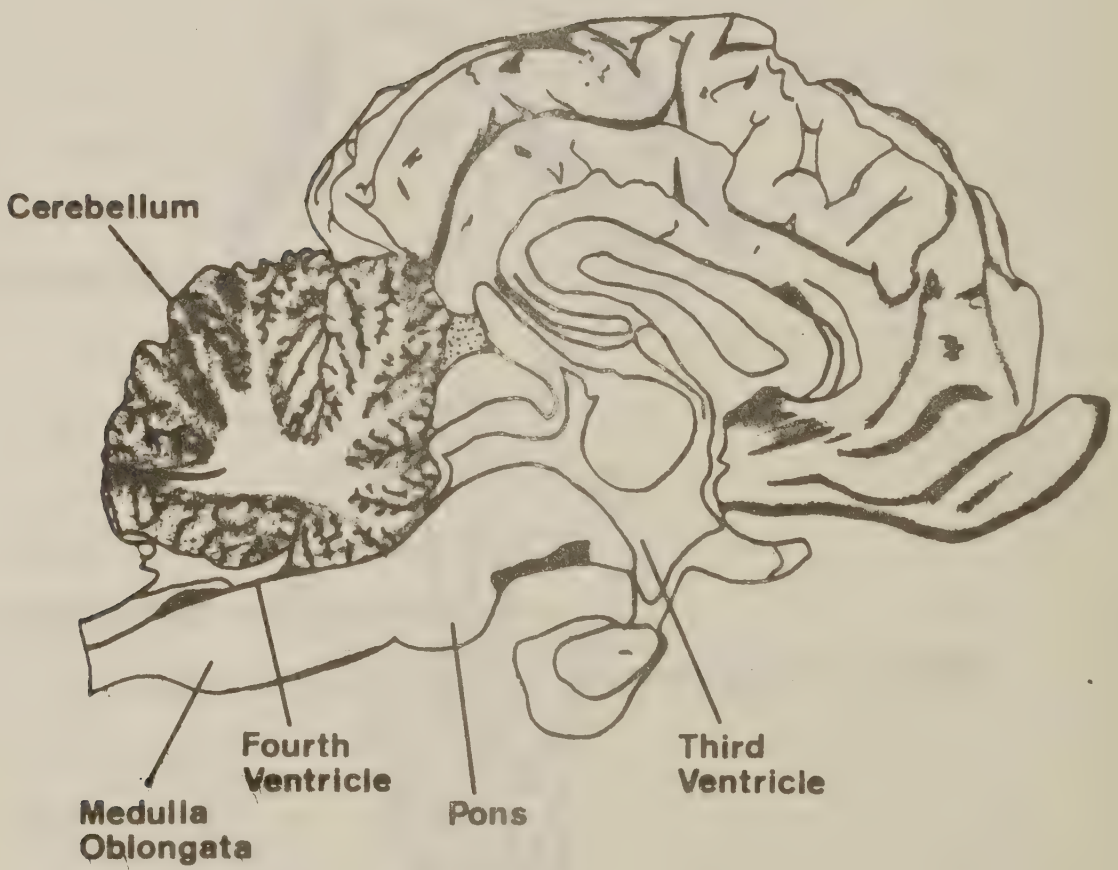
Appendix 1a

DISSECTION BRAIN STEM AND BASAL GANGLIA OF HORSE BRAIN (DIAGRAMATIC)



Appendix 1b

SAGITAL SECTION OF OX BRAIN (DIAGRAMATIC)



SPECIAL SECTION ON HOG CHOLERA

NOTE: In 1978 the Secretary of Agriculture declared the United States of America free of hog cholera. The materials in this section have been collected from authoritative sources.

DIAGNOSIS OF HOG CHOLERA (HC)*

At the time of preparation of this manual (Feb. 1975), approximately a year had elapsed since the last case of HC was diagnosed. It is possible that HC may be regarded as a foreign animal disease in the near future. Since it has been an important domestic disease of swine, a great deal of information on HC is now available in texts and references. Nevertheless, differential diagnosis is now complicated in both the USA and elsewhere by changes in the nature of the disease. A much larger proportion of mild and latent cases rather than the classic fulmonating disease now characterize most outbreaks. In fact, many of the HC diagnoses effected in the course of the latter stages of the eradication program were made on the basis of routine samples taken from herds in which HC was not suspected. For these reasons brief excerpts from authoritative sources on the diagnosis of HC are included in this manual.

I. RECOMMENDED MINIMUM STANDARDS FOR DETECTING HOG CHOLERA VIRAL ANTIGEN BY THE FLUORESCENT ANTIBODY (FA) TISSUE SECTION TECHNIQUE (1)

Specimen Collection and Shipment

Tonsil, spleen and cervical lymph nodes are the tissues of choice in most instances when chronic HC is suspected; two to three inches of the terminal ileum should also be collected. When dead or sick animals are not available for necropsy, tonsil biopsies should be collected. Tonsil biopsies are ideal for this procedure. When other tissues cannot be obtained, one inch ear tips may be submitted. Additional tissues such as kidney, lung, adrenal gland and other lymph nodes may be collected. Each piece of tissue should be placed in separate plastic bags and identified.

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* Prepared by D. H. Ferris

Tissues should be preserved by refrigeration if the tissue can be processed within eight hours after collection. If longer time is required the tissues should be preserved by freezing. Frozen coolant cans provide adequate cooling for refrigerating specimens in transit, but dry ice is necessary for shipping frozen specimens.

Procedure for Conducting Test

A. Trim specimens so the tissue blocks are approximately 1/8 inch thick. These blocks are frozen on a freezing microtome chuck using OCT compound* or water to adhere the tissue to the chuck and to form a supporting matrix collar around the tissues.

B. Cut frozen sections eight microns thick. Tissue sections should be mounted directly on glass slides from the freezing microtome blade. Frosted or plain glass slides may be used, however, all frosted slides have the advantage that the tissue sections are less likely to wash off during subsequent processing.

C. Mounted tissue sections are immediately fixed 10 minutes in reagent grade acetone at room temperature.

D. Fixed sections are flooded with anti HC conjugate and incubated 30 minutes in a moist chamber. Conjugate should cover the entire tissue section. An applicator stick can be used to spread the conjugate over the section.

A simple moist chamber can be made from a 25 x 100 centimeter petri dish by placing two short pieces of an applicator stick and a moist ball of cotton on the bottom of the dish. The slide is placed on the piece of applicator stick to prevent capillary attraction adhering the slide to the petri dish.

*Ames Company, Division Miles Laboratory, Inc., Elkhart, Indiana.

E. Pour off the anti HC conjugate remaining on the slide and wash thoroughly in buffered saline. Excellent results have been obtained by washing actively 10 minutes through three changes of carbonate-bicarbonate buffered saline, pH 9. Clean glassware and buffer should be used for each case to make sure cells are not inadvertently transferred from one slide to another during washing. Carbonate-bicarbonate buffered saline may be prepared as follows:[1]

Preparation of Carbonate Buffer

Solution A - Na_2CO_3	53 gm.
Distilled water to make 1000 ml.	
Solution B - $NaHCO_3$	42 gm.
Distilled water to make 1000 ml.	

Theoretically, a pH of 9.0 should result from mixing 44 ml. of Solution A with 1000 ml. of Solution B, but experience has shown 800 ml. of Solution B (4.2% $NaHCO_3$) and 215 ml. of Solution A (5.3%Na_2CO_3) usually produce a buffer of approximately pH 9.0. The final pH should be checked on a meter and adjusted to pH 9.0.

Preparation of Carbonate-Buffered Saline

Carbonate-bicarbonate Buffer pH 9.0	1000 ml.
Sodium cholride	8.5 gm.

Keep buffered saline tightly stoppered when not in use. Prepare small amounts frequently rather than large amounts infrequently.

F. Rinse the slides quickly in distilled water. This step is necessary to remove buffer salts that would otherwise dry on the slide.

G. Apply coverslip using buffered glycerine to hold the coverslip over the section. (Buffered glycerine is prepared by combining 9 parts glycerine and 1 part carbonate-bicarbonate buffered saline pH 9).

[1] U.S. Department of Health, Education and Welfare. Fluorescent Antibody Technique. 1961. P. 950-51.

Controls

The ever present possibility of unidentified fluorescing material in the tissue section and the possibility of degenerating cells absorbing some of the fluorchrome dye necessitates control sections. Two additional control sections should be cut and fixed in acetone.

Control sections are processed in the same manner as the test sections except control section is flooded with equal parts of HC antiserum and conjugate and the other is flooded with equal parts of normal porcine serum and conjugate. The antiserum or normal serum should be mixed with the conjugate before the solution floods the tissue section. This mixing can be carried out in separate glassware or on the slide adjacent to the tissue section. Subsequent incubation, washing, and cover-slipping are conducted in the same manner as for test section.

It is not necessary to prepare controls from each piece of tissue processed. Routinely control sections are only prepared from tonsilar tissue. However, if tissue other than tonsil such as spleen or lymph node are found to be positive and tonsil is negative, controls should be carried out on the positive tissue.

Known HC positive section should be processed with each case. This serves as a control for the procedure and conjugate as well as the microscope. Tissue sections known to be negative for HC antigen should be processed any time it appears the fluorchrome in the conjugate may be producing undue nonspecific staining.

Interpretation

Typical fluorescing cells containing HC antigen appear light green or apple green with the fluorescence confined to the cytoplasm. Usually several

positive cells occur together to form plaques and individual positive cells are scattered throughout the tissue section. Occasionally only plaques or individual positive cells are observed.

Sections treated with undiluted conjugate have the greatest amount of fluorescence. Fluorescence is usually slightly reduced in the section treated with conjugate plus normal porcine serum. In the section treated with conjugate plus unconjugated anti HC serum fluorescence should be completely blocked or significantly less than that seen in the section treated with normal serum plus conjugate.

In actual practice the most troublesome cases are those in which small focal areas in the tissue section exhibit dull fluorescence, but neither of the control sections exhibit any fluorescence. The cause for this reaction is unknown. However, it may be due to fluorchrome staining degenerating cells. Cases of this type may be interpreted as suspicious of the HC, but the term "positive" should be reserved for cases in which unconjugated anti HC serum reduces or blocks fluorescence.

Microscopic Examination

Darkfield condenser and ultraviolet illumination are necessary. The eye pieces should be 10X. The objectives should be 10X and 25X. The condenser should be high dry darkfield. Oil immersion objective and condenser are not necessary, but may be used according to individual preference. The light source should be a HBO-200 mercury vapor lamp or equivalent. An OG 12 exciter and a blue absorbing barrier filter or the equivalent are satisfactory. An incandescent light source should be utilized to enable switching back and forth from UV to incandescent light. By utilizing both light sources in this manner it can be determined if fluorescence observed is due to light refractive unidentified fluorescing material in the tissue section. Nonspecific fluoresci

material is frequently light refractive under incandescence light whereas fluorescence due to conjugated antibodies attaching to viral antigen ... completely disappers under incandescence light.

II. HOG CHOLERA FLUORESCENT ANTIBODY SERUM NEUTRALIZATION TEST (2)

1. Stock virus is propagated in PK-15 cells and stored at -70°C.

2. Stock virus is titrated in PK-15 monolayers in Leighton tubes. The virus dilution used in the serum neutralization (SN) test should produce an average of 5-6 fluorescent plaques per field when 0.1 ml of virus dilution is inoculated into a Leighton tube culture.

3. Serums to be tested are heat inactivated at 56°C for 30 minutes.

4. Serums may be pooled and tested at one dilution. If pool is postivive, serums in pool maybe tested individually at one dilution or titrated at initial dilutions of 1:2, 1:8, 1:32, 1:128, 1:512.

All virus and serum dilutions are made in Earle's medium containing 0.5% lactalbumin hydrolydate and 25 millimoles $MgCl_2 \cdot 6H_2O$ (5.07 gm/liter).

5. To 1.5 ml of each serum dilution, an equal volume of virus dilution (to give 5-6 plaques /0.1 ml) is added.

6. The serum-virus mixtures are incubated at 37°C for one hour. The following control mixtures are also incubated:

a) virus dilution + hyperimmune serum
b) virus dilution + negative serum
c) virus dilution alone

7. 0.2 ml of each of the above mixtures are inoculated into a Leighton tube culture of a monolayer of PK-15 cells.

8. The inoculated cultures are incubated for 18-24 hours at 37°C.

9. The cover slip cultures are removed, rinsed, fixed in acetone and stained for 30 minutes at 37°C with HC conjugate. The slips are then

rinsed, mounted in glycerol-PBS and read in the fluorescence microscope.

10. A serum dilution is considered positive when there is a 90-100% reduction in fluorescent plaques as compared to the negative serum control.

III. HOG CHOLERA DIAGNOSTIC GUIDE

This is a guide to assist the diagnostician. No single herd or animal should be expected to demonstrate all of the following criteria for diagnosis. Final determination should be based on a composite of information gained through an orderly and comprehensive diagnostic procedure; if a category is applicable, enter the assigned points as the score and check each item observed in determining the final diagnosis. If a category is not applicable, enter "0" as the score. In all cases the professional judgement of the diagnostician is the final determining factor.

1. HISTORY COMPATIBLE WITH HOG CHOLERA (Points assigned - 20) SCORE /____/

__/Sickness in swine not vaccinated against HC.
__/Failure of antibiotic therapy.
__/High morbidity and mortality; few recoveries in unvaccinated swine.
__/Sickness not necessarily confined to any particular age group of unvaccinated swine.
__/One or more pigs dying about time sickness spreads to remainder of herd.
__/Recent additions to herd with additions, or unvaccinated swine exposed to additions, sickening first.
__/Exposure to outside infection through people, equipment, vehicles or animals.
__/Hog cholera outbreaks in neighborhood.
__/Feeding raw or improperly cooked garbage.
__/Improper use of live virus with the vaccinated swine, or unvaccinated swine exposed to those vaccinated, sickening first.

2. CLINICAL SIGNS OF HOG CHOLERA (Points assigned - 10) /____/

__/Temperatures of most of visibly sick swine 105° - 107°. (Temperatures of 10% or 10 swine, whichever is less, of the herd should be taken, including many of the visibly sick.)
__/Sick swine off feed.

2. (Continued)

__/Sick swine having eye discharge, gummed eyelids, or conjunctivitis.
__/A few (often 1 or 2) young sick swine exhibit central nervous system disturbances. (Rarely, older swine exhibit convulsions. The clinician may have to rely on other observers noting convulsions.)
__/Sick swine show purplish discoloration of skin, particularly on abdomen, legs, or ears (may not be apparent on dark skin).
__/When moved from nest, many sick swine: move reluctantly __/ exhibit weaving gait, particularly in hind quarters __/ evidence constipation followed by diarrhea __/ stand with head and tail drooped __/
__/When at rest, sick swine may either:
  __/Pile up, even in hot weather. (This is usually seen fairly early in the course of the disease.)
  __/Lie alone. (This is usually seen in terminal stages of the disease.)

3. PETECHIAE OR ECCHYMOTIC HEMORRHAGES (Points assinged - 20) /____/

__/Petechiae or ecchymotic hemorrhages are most consistently observed in the following: larynx __/ epiglottis __/ kidney __/ urinary bladder __/

4. PERIPHERAL HEMORRHAGE OF LYMPH NODES (Points assigned - 20) /____/

__/This type of hemorrhage assumes greater significance when it appears in nodes of more than one body region. Hemorrhages observed in some nodes may be so severe as to appear diffuse rather than peripheral. As a minimum, the following nodes should be examined: submaxillary __/ cervical __/ hepatic __/ mesenteric __/ renal __/ suprainguinal __/

5. INFARCTS (Points assigned - 20) /____/

__/Spleen-Infarcts are most significant in the spleen.
__/Colon or Cecum-Characteristic "button ulcers" are not always confined to the area near the ileo-cecal valve, but may be found in adjacent areas of colon or cecum.
__/Gall Bladder-Infarcts of the gall bladder may appear to be hemorrhages.
__/Tonsil-Tonsillar infarcts often appear as small abscesses. In HC, the tonsil may also show purplish discoloration.

6. RIB CHANGES (Points assigned - 10) /____/

__/These changes occur at the epiphyseal area of the junction of the rib and cartilage near the sternum, and are most pronounced in the sixth to eighth ribs. Abnormal calcification at the costo-chomdral junction results in a widened or irregular epiphysis, hemorrhagic epiphyseal line, or (in chronic cases) a tranverse line of very dense bone between normal bone layers just proximal to the epiphysis.

7. LEUCOPENIA DEMONSTRATED (Points assigned - 30) /____/

__/White blood cell counts should be made from acutely sick swine over six weeks of age. To demonstrate leucopenia, one or more counts should be less than 10,000/ml. The possibility of leucopenia cannot be eliminated until counts are made from at least six such swine with all ranging over 10,000/ml.

8. BRAIN HISTOPATHOLOGY POSITIVE (Points assigned - 50) /____/

__/A laboratory function involving histopathological search of brain tissue for characteristic vascular lesions.

9. FLUORESCENT ANTIBODY TEST POSITIVE (Points assigned - 70) /____/
__/Tissue Culture
__/Tissue Section

10. ANIMAL INOCULATION POSITIVE (Points assigned - 70) /____/

__/This procedure is not utilized for routine diagnosis, but is reserved for those occasions where diagnosis cannot be made otherwise, or when it is essential that initial diagnosis be further confirmed.

11. TOTAL SCORE /____/

GUIDE FOR HOG CHOLERA DIAGNOSIS

POSITIVE - 100 or more Points - In areas engaged in final eradication procedures, a positive diagnosis must always include at least 50 points in items 1 through 6 and one or more positive findings in items 7 through 10.

SUSPICIOUS - 20 - 90 Points - Further work should be done to establish the presence or absence of HC.
10 or more Points - In areas engaged in final eradication procedures, a score of 10 or more should be regarded as suspicious.

NEGATIVE - 0 - 10 Points

IV. INTERNATIONALLY RECOGNIZED PROCEDURES FOR THE DIAGNOSIS OF HOG CHOLERA (4)

1. Clinical data	Observations	Suspicion	Confirmation	Delay
a. epizootology	Contagiousness Subjects of all ages.	+		
b. Signs	Fever Cutaneous lesions Cough-diarrhea Neurological signs	++		
c. Macroscopic lesions	Disseminated hemorrhages or necroses (skin-lymph nodes-heart-lungs-intestines-kidneys-bladder)	+++	C	
2. Laboratory investigations:				
d. Microsc. exam.	Material Nervous system* (non-purulent panencephalitis)	+++		48 hrs.
e. Immunofluorescence			C	
Cryostat	Tonsils *			2 to 3 hrs.
Cell culture	Spleen, mes. ganglia *			24 to 72 hrs.
f. END test **	idem		C	8 days
g. Rabbit inoc.	Spleen *	+++		15 days
h. Agar precip.	Pancreas *		C	24 to 48 hrs.
i. CF test	Liver-spleen *		C	24 hrs.
j. Antibodies		+ or C		
SN (PAV-1 strain)	Convalescent serum*			8 days
SN (HEIC test) ***				8 days
FA tests		+		2 to 3 hrs.
Agar Precip.				24 to 48 hrs.
k. hyperimmune pig inoc.	Spleen *	(differentiation from ASF)		8 to 12 days

* Material to be removed for transmission to the laboratory where the entire cadaver can not be sent.

C Confirmation of HC

** Exhaltation of Newcastle Disease virus test.

*** Test employing "ADL" cell culture adapted strain of HC virus (Nat. Vet. Lab. Kokubunje, Tokyo, Japan).

References

(1) Excerpts from: Bedell, D., McDaniel, H. A., Clark, C. D., Grey, A. P., Aikin, J. 1968. RECOMMENDED MINIMUM STANDARDS FOR DETECTING HOG CHOLERA VIRAL ANTIGEN BY THE FA TISSUE SECTION TECHNIQUE U.S. Livestock Sanitary Assoc. Proceedings, 72nd Annual Meeting. (For official USDA use).

(2) Excerpts from: Smithies, L. K. 1975 Senior Virologist, Wisconsin Animal Health Laboratory and USDA State-Federal Cooperative Program (Modification of protocol from Carbrey, E. A., Stewart, W. C. et. al., 1969 JAVMA 155:2201-2210)

(3) GUIDE FOR HOG CHOLERA DIAGNOSIS furnished by APHIS to HC Diagnosticians

(4) Table on HC Diagnostic Procedures adapted from Rabot, L. G. 1971. PROPERTIES OF THE VIRUS OF CLASSICAL SWINE FEVER AND DIFFERENTIAL DIAGNOSIS OF CLASSICAL AND AFRICAN SWINE FEVER. Page 48, Commission of the European Communities, Directorate-General for Agriculture.

SPECIAL REFERENCE HANDBOOK SECTION

CONTAGIOUS EQUINE METRITIS

Prepared by: J. S. Walker

FORWARD

The information contained in this handbook was obtained from the references listed and personal communication with investigators, veterinarians and horse breeders in England and Ireland during an investigative visit by a team from the U.S. in September 1977. Much of the information is of an unpublished nature at this point.

Certain portions, i.e., gynecological veterinary examination of mares and stud hygiene are taken directly from the David and Powell article (Veterinary Record 101.189) because it is the only record and experience available on the subject. Members of the team that investigated the situation in England and Ireland included Doctors R. C. Knowles, APHIS; J. Moulthrop, APHIS; J. S. Walker, PIADC; and W. R. McGee, American Association Equine Practitioners.

More material is included in this handbook section than in other sections of the manual because of the present scarcity of published data on the disease.

CONTAGIOUS EQUINE METRITIS

I. Identification of disease:

A. Definition.-- Contagious equine metritis (CEM), also termed contagious metritis 1977, is a highly contagious acute veneral disease of horses that severely effects breeding operations and fertility.

B. Etiology.--The causative organism is a bacterium that has not been classified to date, but has characteristics of the genus Moraxella and its DNA base composition is close to Hemophilus. The non-motile coccobacillus is gram-negative. It is catalase and oxidase positive and in addition, is characterized by being very unreactive or completely negative on standard bacteriological differential tests commonly used. The medium used for primary isolation is chocolate agar incubated at 37C in an atmosphere of hydrogen or air containing 5 to 10% CO_2. When Eugon agar is used as a base for the chocolate agar, the colonies can be observed after 24 hours incubation. The colonies are shiny, smooth, butyrous and slightly grey. After prolonged incubation, beyond 72 hours, the colonies appear white, raised and glistening. An aid to isolation of this organism from field specimens is its resistance to streptomycin.

Unpublished data indicates that after 7 passages or so in the laboratory, the organism starts to lose virulence for the horse and dependency on CO_2 for *in vitro* growth. Both of these characteristics apparently can be restored by *in vivo* passage.

C. History.--The disease was first officially reported in the early summer of 1977 in England where 29 thoroughbred horse stud farms around Newmarket were affected causing some of them to close. The British have estimated that the economic losses to the thoroughbred industry during the 1977 breeding season totaled at least 30 million dollars. To date, the disease has only been reported in thoroughbred horses. Information recently obtained revealed that CEM occurred on at least 4 stud farms in Ireland during the 1976 spring breeding season and also during their fall breeding season of 76. It recurred in Ireland again during the 1977 breeding season, as well as in France. Although unconfirmed, there are indications that CEM may have occurred in France prior to 1976. CEM occurred in Australia during the breeding season of 1977 and is traceable to mares bred in Ireland during the 1976 fall breeding season.

It is of interest that the three countries, Ireland, England and France, entered into a tripartite agreement in the early 1970s which allowed particularly free movement of horses between the three countries without quarantine and with minimal veterinary inspection.

As of September 1977, the U.S. and Canada have placed a ban on the importation of all equidae, except geldings, weanlings and yearlings, from Ireland, U.K., France and Australia.

II. Signs:

A. Clinical features.--To the clinician or stud owner, the first

noticeable indication that anything is wrong is that suddenly every mare being covered by a particular stallion starts coming back into premature estrus and is what the horse clinicians term "dirty" on vaginal examination. Upon routine aerobic culture, no specific organism of importance is isolated, except maybe *Proteus sp.* and other secondary invaders or opportunistic organisms during certain phases of the infection. However, using special culture techniques, the gram negative coccobacillus can be isolated. The role that *Proteus sp.* and other opportunistic organisms plays in the total disease picture has not yet been fully defined.

The infection is characterized by an endometritis associated with cervicitis and vaginitis. A copious mucopurulent discharge is sometimes seen coming from the vulva, smearing the buttocks and matting the tail. However, the severity varies from almost a complete sloughing of the endometrium to mares that appear almost clinically normal on examination except that they are in premature estrus.

The stallion is completely normal upon examination and shows no clinical signs of disease, yet they may remain carriers and transmit the disease for extended periods of time. One stallion in Ireland contracted the disease in the spring of 1976, was treated with ampicillin during the early summer and still infected mares during the fall breeding season of 1976. Thus, the stallion in CEM appears to play the identical role in the spread of the disease that the bull does in Trichomonas.

Little is known at this time about chronic asymptomatic carriers; however, they do occur. To illustrate this point, there are two mares in England that are 200 plus days in foal from which the gram-negative coccobacillus can still be isolated. They were infected early in 1977, treated, cleared up clinically and conceived when rebred in the early summer. However, the gram-negative coccobacillus has been isolated from practically every swab during the pregnancy. Another known example is a barren mare in England that had a severe case of the disease early in 1977, was treated, but not rebred. She became completely normal on clinical examination and was negative for the organism on two consecutive estrus periods, yet reverted to positive on the third estrus cycle.

The true incidence of abortions that occur is unknown at this time. However, it is known to occur and generally within the first 60 days of pregnancy because mares that were judged in foal on the 3 and 6 week examinations came up "empty" on 90 and 120 day examination.

B. Incubation period.--The incubation period varies from 2 to 14 days under natural conditions. Although experimental work is preliminary at this stage, it appears that the average incubation period is 2 to 4 days for the appearance of exudate in the vaginal tract with maximal inflammation of the cervix and vaginal mucosa occurring around 5 to 10 days postinoculation.

III. Pathologic changes:

A. Post-mortem lesions.--Very few mares have been examined, thus the description given below is subject to change. The gross lesions varied from a slightly enlarged uterus containing a small amount of greyish fluid to almost a normal appearing uterus. More extensive changes have been reported; however, they are probably the results of secondary infections. Thus, it appears that the gross pathology varies greatly as do the clinical signs.

B. Microlesions.--The following is based on endometrial biopsy material. Infected mares showed changes of focal luminal epithelial hyperplasia and very marked degenerative changes at sites immediately below the luminal epithelium at the junction with the stratum compactum. Some epithelial cells showed advanced cytoplasmic and nuclear degenerative change with sub-epithelial "vacuoles" containing amorphous debris sometimes giving the impression of "inclusion bodies". There was often a supra-epithelial amorphous layer containing polymorphonuclear leucocytes. However, the predominant cellular infiltration in the stroma was mononuclear, occurring densely in some areas. Limited examination of experimentally infected mares essentially confirms the above in that the lesions are minimal and confined to the endometrium.

IV. Diagnosis:

A. In the field.--The clinical disease will appear during the breeding season which in the Northern Hemisphere is from February through mid-July.

The presumptive diagnosis is based on several mares covered by a particular stallion coming back into premature estrus which appear "dirty" upon vaginal examination. See clinical signs for complete description of the clinical findings.

B. Laboratory.-

(1) Endometrial smear. During active infection, large numbers of polymorphonuclear leucocytes (PMNs) can be found in uterine lumen and in vaginal exudate. If PMNs are found on the smear stained either with Leishman or the rapid trichrome stain, they are indicative of active uterine infection even if bacteriological swabs are negative. Gram-stained smears are also useful, but due to the presence of several types of gram-negative organisms in the genital tract, a positive identification of the gram-negative coccobacillus can not be made from a smear.

(2) Swabs for culture from mares. Cervical swabs for bacteriological examination are best taken at estrus when the cervix is relaxed. Experienced clinicians of the disease have observed that even at estrus the cervix is difficult to penetrate in infected mares. Ideally, the swab should be guarded to avoid contamination, be rigid enough to penetrate the cervical canal and suitable to be easily deposited into transport medium; in addition, a urethral and clitoral swab should always be taken. The clitoral swab may be particularly important in suspect carriers of CEM. Stuart's or Amies transport medium should be used. Laboratory tests

indicate that the organism can survive in them for five days at 20C. It is nevertheless important that swabs in the transport medium be transported to the laboratory as quickly as possible.

(3) Swabs for culture from stallions. If several mares from a particular stallion are affected with the clinical signs, the stallion should also be swabbed for culture. The swabs should be taken from the penile sheath, the urethral fossa and the urethra. Of the three sites, the urethral fossa is the most important. The swabs should be handled as outlined above for the mares.

(4) Laboratory culture. Upon arrival at the laboratory, the swabs are streaked on chocolate agar containing 200 to 400 µg/ml streptomycin as well as regular blood agar plates. One plate of each is incubated aerobically and one each in 5% CO_2 at 37C for 24 hours. The coccobacillus will grow only on the chocolate agar plate in 5% CO_2; thus both blood agar plates and the aerobic chocolate agar plates should be negative for the coccobacillus colonies. Typical appearing colonies are picked with a fine glass rod and the oxidase test performed; if the oxidase test is positive, a gram stain is performed as well as the catalase test using similar appearing colonies. If all three are positive, a positive laboratory diagnosis is given. If any one of the three tests are negative , Cooked Meat Medium is inoculated with a suspect colony and incubated overnight at 37C. From this, new plates are streaked, incubated

at 37C aerobically in 5% CO_2 for another 24 hours and the three tests repeated.

(5) Serology. To date no serological tests are available.

C. Differential diagnosis.-- Although the clinical signs, vaginal examination and smear examination may lead one to suspect CEM, a final diagnosis can only be made upon isolation of the causative organism. The two most common vaginal infections in mares that can be confused with CEM are those caused by *Klebsiella sp.* and *Proteus sp.* and the latter organism may be involved in CEM as a secondary invader.

V. Prognosis:

It appears that a stallion once infected will transmit the disease to every mare he covers. The infected mares generally will remain barren until the next breeding season because of the time required to treat them and assure that they remain negative for the organisms through a minimum of three estrus cycles. Data from Ireland indicates however, that conception rates for the mares and breeding rates for stallions both return to normal the following breeding season if they have been cleared of the infection.

VI. Epizootiology:

A. Geographical distribution.-- see history

B. Transmission.--The spread of the disease is primarily by venereal transmission, but it can be spread by personnel and their equipment in the handling and examination of infected mares. Routine veterinary examination

presents a particular hazard and rigid aseptic techniques must be employed during genital examination of all mares. Stud personnel who handle the genitalia of mares at teasing, washing up of the genitalia of mares and stallions in the covering yard or stalls, or those who assist at veterinary examination can also transmit the disease.

C. Host range.-- It is assumed that all equidae are naturally susceptible and several types of ponies have been experimentally infected. However, CEM has to date, occurred only in thoroughbreds under natural conditions. Experimental work in Ireland has failed to produce the disease in cattle, sheep and swine. Laboratory animals have not been tested to date.

VII. Control and eradication:

A. Preventive measures.--

(1) Breeding stock from affected countries should not be allowed into countries that are free of the disease.

(2) Any mares or stallions currently in a country that is free of the disease but that were used or covered in one of the affected countries (Ireland, U.K., France, Australia) during the 1977 (Ireland and France 1976 and 77) breeding season should be swabbed and cultured prior to the next breeding season. Pregnant mares should only have a urethral swab made.

(3) Gynecological veterinary examination of mares and stud hygiene: Because of the highly contagious nature of the disease, careful attention should be paid to hygiene when mares are examined.

a. Disposable gloves, one pair per mare, should be used by the veterinary surgeon and his assistant. A washable gown and boots should be kept at each large stud farm, but veterinary surgeons who examine only the occasional mare should take great care to wash all protective clothing in chlorhexidine before leaving the premises.

b. A thorough cleansing of the vulva using disposable swabs and a suitable antiseptic is a must.

c. A separate sterile speculum should be used for the examination of each mare. Instruments should be sterilized by boiling or autoclaving.

d. Separate equipment should be provided on each stud farm, particularly if an outbreak is occurring in the area. If this is not possible, it remains the responsibility of the veterinary surgeon to ensure that the equipment is sterile.

e. Stud farm personnel, whenever they handle the genital area and tails of mares should wear disposable gloves, one pair per mare. The attending veterinarian must emphasize the importance of hygiene to all stud personnel and explain the need for it.

VIII. Public health aspects:

There is no known transmission to man as of this date. However, caution should be practiced because little is known about the organism and disease at this time including the original source of the organism or its classification.

IX. Therapy:

A. Treatment of infected mares.--Laboratory tests indicate that the gram-negative coccobacillus of CEM is sensitive to a wide range of antimicrobial agents. Experience obtained during the 1977 outbreak indicates that intensive parenteral treatment should be undertaken, although a single course of treatment does not eliminate the disease in every case. It appears that 2-4 grams of ampicillin administered IM; BID or TID (total of 6-8 grams daily) for 10 to 14 days along with intra-uterine irrigation is the treatment of choice. In addition, recent experimental work in Ireland indicates that parenteral neomycin at the dose rate of 2 grams daily for 5 days when combined with intra-uterine infusions, is also effective. Although not fully supported by experimental work to date, less intense therapy appears to induce a carrier state in certain mares. The parenteral therapy should be supplemented by intra-uterine irrigation with nitrofurazone, ampicillin, benzyl penicillin or a combination of nitrofurazone and neomycin solution daily for a period of 3 to 5 days with particular attention being taken to include a thorough cleansing of the clitoral fossa.

B. Treatment of infected stallions.--If it is confirmed that the stallion is transmitting the CEM organism, then covering should cease immediately. The penis and prepuce should be thoroughly cleansed with chlorhexidine (1 oz/gal) and treated with nitrofurazone once daily for at least 3 days or with 3 treatments over a 5 day period, with particular attention

being given the urethral fossa. In conjunction with local treatment as above, both oral treatment with nitrofurantoin for five days or ampicillin for 10 days appear to be successful. Further treatment may be necessary depending on the results of bacteriological tests and trial breeding if the economics justify it. In any case, covering of mares should not begin until three consecutive negative samples have been obtained. Sampling should begin one week after treatment has ceased, with an interval of not less than seven days between samples. On each sampling, swabs should be obtained from the sheath, the urethral fossa, the urethra and the preejaculatory fluid and submitted to an official USDA laboratory in Stuart's transport medium.

These methods of treatment may be modified as further information becomes available.

Suggested Reading:

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FORWARD TO DIAGNOSIS SECTION

The enclosed information on the diagnosis of contagious equine metritis (CEM) is written as a result of four months' research using nineteen mares. These mares are still under observation and much information will be gleaned from them. In addition, this information includes some of the pertinent published literature and a guideline in the preparation of reagents for disease diagnosis. When the information is complete, it will provide a "cookbook" to be followed in the diagnosis of the diseases. Presently it contains the meat of today's information and is only in draft form. We must realize that research on CEM is continuing and that information is evolving. We are providing you with today's information and will send a final writeup as soon as it is available.

A. H. Dardiri
Laboratory Chief
Diagnostic Research

DIAGNOSIS OF CONTAGIOUS EQUINE METRITIS

Contagious equine metritis (CEM) is a newly reported venereal disease of breeding horses. It has never been diagnosed in the USA, but appeared in France, England, Ireland and Australia.

Any disease which affects any segment of the animal industry and causes possible economic loss will always be considered important and usually generate research at all levels. As a rule, diagnostic preparedness precedes other areas of research activities, and CEM is no exception.

Like any other disease, a greater chance of diagnosing CEM is achieved when the clinical disease profile is understood and the optimal sampling time is known. In order to insure that the laboratory scientist will proceed expeditiously in arriving at a diagnosis and make an adequate evaluation of the laboratory finding, it is necessary that the clinician consider the following:

1. Collect the specimens as asceptically as possible from the anatomic sites where the pathogen is most likely to be found.
2. Provide the laboratory with the epizootiological and clinical history.
3. Exercise every precaution in labeling, packaging, storage and transportation of the specimens collected from the animals to the laboratory, and to be sure the suspect organisms remain viable.

Collection and Handling of Specimens

1. Diagnoses of infections in mares and stallions are made by obtaining swabs from: a) uterus, b) cervix, c) urethra, d) clitoral fossa, and e) penile sheath.

2. Specimen from suspected equine should be inoculated on selective media when collected and incubated under 5-10% CO_2 at 37°C; however, field specimens may be transported under adverse circumstances over a considerable distance for as long as four days. Therefore, the specimen on swabs are placed in suitable transport media (Stuarts or Aimes) containing bacterial inhibitors.

Diagnosis

1. Field - Premature estrus

   Acute genital tract inflammation only in mares

   Vaginal discharge

   Signs appear during breeding seasion - Northern Hemisphere-February-mid-July

2. Laboratory

   Active infection

   A. Large number of polymorphonuclear leukocytes in uterine swabs and exudate

   B. Swab cultures from mares

      1) Uterus
      2) Cervix
      3) Urethra
      4) Clitoral fossa

C. Swab cultures from stallions

1) Penile sheath
2) Urethral fossa
3) Urethra

Maintain swabs in transport media

D. Laboratory cultures

3. Transportation of Specimens

A. Use sterile non-toxic swabs.

B. After the swab is used to obtain a specimen, insert it deep into the transport medium in a vial. Cut off the stick and replace the cap tightly.

Isolation procedure for CEM coccobacillus

CEM swab inoculated in:

1. Eugon chocolate agar

Inoculate a plate

Incubate in CO_2 at 37°C

Pick suspect colonies at 48 hrs

Streak on 1, 2, 3, 4 plates, Gram stain

2. Tryptose chocolate agar

Inoculate a plate in CO_2 at 37°C

Pick suspected colonies at 48-72 hrs.

Streak on 1, 2, 3, 4 plates, Gram stain

3. Blood Agar

Inoculate a plate

Incubate aerobically at 37°C

Examine at 24 and 48 hrs

a) Bacterial contaminants appear in 24 hrs

b) If no growth in 48 hrs incubate in CO_2 at 37°C

4. EMB or MacKonkey agar

Inoculate a plate

Incubate aerobically at 37°C

5. Eugon broth with 1 µg. Hb

Insert swabs into broth tube

Incubate in CO_2 at 24, 48 hrs

Streak 1, 2, 3, 4 media

Bacterial contaminant associate with growth of CEM coccobacillus on chocolate agar, blood agar, and broth.

a) Gram negative rods

E-coli, Klibsiella, Proteus, Pseudomonas sps

b) Gram positive rods

??? Lactobacilli sp

c) Gram positive cocci

Streptococci sp

Micrococci sp

d) Yeast

SCHEMATIC CHART FOR ISOLATION OF CEM COCCOBACCILLUS FROM SWABS OF UTERUS, CERVIX, URETHRA, CLITORIS FOSSA AND PENAL SHEATH

(1) EUGONS CHOCOLATE AGAR	(2) TRYPTOSE CHOCOLATE	*(3) BLOOD AGAR	(4) EMB MACCONKEY	EUGONS BROTH 1 µg/ML HEMOGLOBIN
CO_2 INCUBATION	CO_2 INCUBATION	AEROBIC INCUBATION		CO_2 INCUBATION
48 HRS	48--72 HRS	48 HRS		24 & 48 HRS STREAK BROTH ON NO. 1, 2, 3, 4 PLATES

PICK SUSPECT COLONIES
STREAK 1, 2, 3, & 4 MEDIAS

* BLOOD AGAR WITH BASE 1, 2 MEDIA

JANUARY 1958

CEM Bacterial Culture Growth Interpretation

1. Eugons chocolate agar

   CEM and bacterial contaminant colonies may be similar in size, usually CEM colonies are smaller

2. Tryptose chocolate agar

   CEM colonies tiny <0.2 mm

   Contaminant colonies are larger

   a) Pick tiny colonies and streak on agar plates of 1, 2, 3, 4 media

   b) Gram stain colonies

   c) Colonies of contaminants appear at 24 incubation on blood EMB or MacKonkey agar plates

(See chart Page 5)

Growth of CEM coccobacillus colonies on Agar Plates

Time	Eugon chocolate (CO_2)	Tryptose chocolate (CO_2)	Blood
24 hrs	+	-	-
48 hrs	0.2 - 1 mm	<0.2 mm	-
96 hrs	1 mm or >	0.2 mm	-

Growth of CEM coccobacillus colonies in Broth

Hours	*Eugons Broth	Eugons Broth with Hb 1 µg	Eugons Broth with Hb 10 µg	Cooked Meat
24	-	±	-	±
48	-	+	-	+
72	-	++	+	++
96	+	++	+	++

* only 50 - 75 % of the cultured colonies show growth as judged by turbidity

CEM

Clinical Disease Features

Mares.

1. Premature estrus of covered mares
2. Metritis

   Cervicitis

   Vaginitis
3. Copious mucopurulent-discharge
4. Severity varies

Stallions

No signs

Carriers - not determined

Mares - clear up and conceive

- clear up and not conceive
- clear up and revert

positive CEM agent isolation at estrus period

Abortion usually within 60 days of pregnancy

Incubation period

2 - 14 days Natural infection

2 - 4 days Experimental infection

Isolation of CEM organism from the genital tract following experimental infection. (Platt et al 1978 Vet. Rec.)

Day Post Infection	Clinical Signs	Bacterial Finding		
		Cervix	Urethra	Clitoris
2	Present	+	+	+
13	"	+	0	+
23	"	+	+	+
41	Suspicious	+	+	Nt
55	Absent	0	+	+
72	"	0	0	+
80	"	0	0	+

+ Culture Isolation

\- " "

Nt not done

Media for CEM Coccobacillus Isolation

1. Chcolate agar plates
   a. Eugon agar (BBL)
   b. Tryptose blood base agar (DIFCO)
2. Blood agar (base a or b)
3. EMB agar

   or

   MacKonkey agar
4. Broth
   a. Eugon (BBL) supplemented with
      1 µg/ml hemoglobin
   b. Cooked meat (BBL)

A. Eugons chocolate agar

Eugon-agar	45.µg
Distilled water	1 litter

1. Suspend agar, autoclave 121 C, 15 min
2. Cool to 50°C, add 100 ml defibrinated sheep or horse blood
   mix gently
3. Place medium in a water bath at 75°C for 10 - 15 min. until chocolate in color
4. Cool to 50°C, add 300 µg/ml Streptomycin, mix thoroughly and gently, remove broth using negative pressure
5. Stir while pouring 20-25 ml/plate
6. Store at 5°C and use within 10 days

B. Tryptose blood agar base

Follow above method

Broth

Eugon (BBL)	30 g
Distilled Water	1 liter
Hemoglobin (sheep) Sigma 2 x crystalized	1.0 mg

Dissolve, autoclave 121 C, 15 min.

Cool to room temperature

add streptomycin 300 µg/ml

Dispense asceptically in 10 ml amounts in sterile screw capped tubes

Store at 5°C

Amies transport medium

Sodium chloride	8.0 g
Potasium chloride	0.2 g
Calcium chloride	0.1 g
Magnesium chloride	0.1 g
Monopatasium phosphate	0.2 g
Disodium phosphate	1.5 g
Sodium thioglycotate	1.0 g
Charcoal, fine powdered	10.0 g
Agar	3.6 g
Distilled water	1.0 liter

1. Mix and heat gently to dissolve soluable ingrediants
2. Sterilize at 121 C for 15 min.

3. Cool to 50°C, and add 300 µg/ml streptomycin, mix gently
4. Dispose in small screw-capped vials filled within 5 mm of the top

Invert vials just before gelation to suspend the charcoal evenly.

Retighten caps if necessary.

Stuart transport medium (Cary and Blair)

Agar	4.00 g
Distilled Water	1.00 liter
Heat until dissolved and add while hot:	
Sodium chloride	3.00 g
Potassium chloride	0.20 g
Disodimine phosphate anhydrams	1.15 g
or disodium phosphate 12.H_2O	(2.9 g)
Monopotassium phosphate	0.20 g
Sodium Throglycolate	1.00 g
Calcium chloride 1% aqueus, freshly prepared	10. ml
Magnesium chloride .6H_2O 1% aqueus	10. ml

Final pH 7.3

1. Stir until dissolved. Add 10g pharmaceutical neutral charcoal
2. Autoclave 121 C., 20 min, cool to 50 C, add 300 µg/ml streptomycin, invert-tubes prior to solidification in order to distribute the charcoal evenly
3. Dispense 5 to 6 ml per 13 by 100 mm screw capped tube, stirring to keep the charcoal evenly suspended (avoid cooling or jelling)
4. Store in refrigerator
5. Avoid prolonged heating at any time

Gram stain

A. Crystal violet solution

Solution A:

Crystal violet (certified)	2 g
Ethyl alcohol .95%	20 ml

Solution B:

Ammonium oxalate	0.8 g
Distilled water	80.0 ml

Mix solution A and B. Store for 24 hours before use. Filter through paper into staining broth.

B. Gram iodine

Iodine	1 g
Potassium iodide	2 g
Distilled water	300.0 ml

Grind the dry iodine and potassium iodide in a mortor. Add water, a few milliliters at a time, and grind thoroughly after each addition until solution is achieved. Rinse the solution into an amber glass bottle with the remainder of distilled water.

C. Decolorizers

1. Ethyl alcohol 95%: slowest agent
2. Acetone - alcohol: intermediate

   (95% ethyl alcohol, 100 ml, acetone, 100 ml)

With experience, any one of the 3 decolorizing agents will yield good results.

D. Counterstain (stock solution)

Safranine O (certified)	2.5 g

Ethyl alcohol, 95%	100.0 ml
Working solution	
Stock solution	10 ml
Distilled water	90 ml

Staining procedures:

Flood dried smear with crystal violet solution and let stand for 1 min. Wash smear lightly and gently with tap water and drain off excess water. Flood smear with iodine solution and let stand for 1 min. Wash with tap water and decolorize until the solvent flows colorlessly from the slide. Wash briefly with tap water. Counter stain with safranin for 10 seconds. Wash briefly with tap water, blot dry, and examine.

Gram positive organisms are blue, gram negative are red.

Methods of taking and staining Endometrial Smears:

A. 1. Obtain swab from uterine lumen
   2. Rub cotton tip with reasonable pressure
   3. Make several smears on clean glass slides
   4. Allow to dry in air
   5. Stain dry smears with Leishman's stain

B. Leishman Stain
   1. Pour the undiluted stain on the unfixed film and allow to act for one minute.
   2. By means of a pippette and rubber teat add double the volume of distilled water (pH 7.0) to the slide, mixing the fluids by alternately sucking them up in the pippette and expelling them. Allow the diluted stain to act for twelve minutes.

3. Flood the slide gently with distilled water, allowing the preparation to differentiate in the distilled water until the film appears bright pink in color - usually about half a minute.
4. Remove the excess water with blotting paper and dry in air.

C. Interpretation of Smears

Polymorphonuclear leucocytes (PMNs) can be readily recognized as pale staining cells with lobulated dark staining nuclei, examples can be seen in Fig. 1. The epithelial cells have pale blue staining cytoplasm and oval darker staining nuclei.

Oxidase test

Reagent A: 1% solution of tetramethyl -p- phenylenediamine dihydrochloride

Prepare daily

or

Refrigerate for not longer than a week

Method Remove a colony with a sterile platinum loop

Rub colony on a filter paper impregnated with the oxidase reagent

Reaction The moist paper impregnated with bacteria turns purple within seconds

use young cultures.

Catalase test

The organism should be from a colony of the organism to be tested.

To test for catalase, pour 1 ml of a 3% solution of hydrogen peroxide over the growth. The appearance of gas bubbles indicate a positive test.

An alternative method is to emulsify a colony in one drip of 30% hydrogen peroxide on a glass slide. Immediate bubbling is indicative of a positive catalase test.

Extreme care must be exercised if a colony is taken from a blood-agar plate.

The enzyme catalase is present in red blood cells and the carry over of blood cells with the colony can give a false positive reaction.

A.H. Dardiri
January 1978

APPENDIX A

CLINICAL SIGNS OF CEM

A. Stallion

1. Asymptomatic
    a. No lesions
    b. No exudate
2. Breeding history of mares may incriminate stallion
3. May culture CEM bacterium from urethral fossa, urethra, or penile sheath

B. Mare

Signs vary from acute to chronic and depend upon such factors as: age, number of previous pregnancies, virulence of organisms, stage of reproduction cycle, host resistance, etc.

<u>First Indication May Be</u>:

1. Failure to conceive
2. Premature estrus

<u>Acute case</u>

1. Vaginal discharge 2-6 days following exposure and lasting for 10-15 days
    a. Copious in amount
    b. Color-opaque
    c. Character: tenacious and mucoid
2. Vaginitis
    a. Hyperemic
    b. May be pool of exudate on floor of vagina

3. Cervicitis

    a. Hyperemic

    b. Edema

    c. Exudate adherent to

Subacute

1. Minimum amount of exudate
2. Cervicitis
3. Mild to inapparent vaginitis

Chronic or Asymptomatic

No visible changes

May recover organisms from clitoral fossa

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APPENDIX B

DIAGNOSIS OF CEM

I History

History should include pertinent information regarding such things as:

A. Age of mare

B. Breeding history

1) Stallion bred to

2) Similar problem in other mares

3) Possible abortion

4) Premature estrus

5) Status of other mares bred to same stallion

C. Previous cultures

II Clinical Signs

A. Premature estrus following breeding

B. Copious discharge - 2-8 days post-breeding

Discharge usually opaque, tenacious and non-odorous

C. Presence of a cervicitis

Edema and hyperemia of cervix

D. Mild vaginitis - may be so mild, it could be overlooked

III Culture of the CEM organism in mares (best method)

A. Best time to culture - estrus

B. Proper technique is paramount for obtaining a suitable culture (minimum contamination)

C. Swab should be taken from either cervix or uterus in acute or subacute case

D. Swab taken from urethra and/or clitoral fossa in chronic case

IV Culture of the CEM organism from stallions

A. Swab taken from the following areas:

1) Sheath

2) Urethra

3) Urethral fossa

V Smear of Vaginal Exudate

A. Stain with Leishman stains

Look for polymorphonuclear leucocytes

B. Stain with Grams stain

Look for gram negative coccabacillus

APPENDIX C

DIFFERENTIAL DIAGNOSIS OF CEM

Other Courses for Infertility or Infection in Mares

A. Bacteria

1) Streptococcus zooepidemicus (B hemolytic strep)>50%

2) Escherichia coli 17%

3) Klebsiella sp. 8%

4) Staphylococci 8%

5) Corynebacterium sp. 3%

6) Shigella equili 3%

7) Proteus sp.

B. Mycosies (fungi)

C. Pyometra

D. Infertile stallion

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APPENDIX D

TRANSMISSION OF CEM

A. Stallion

   Venereal infection (most common way)

B. Teaser Studs

   Spread infection by way of muzzle

C. Man

   1. Contaminated instruments

      a. Vaginal speculum

      b. Culturing equipment

      c. Gloves, etc.

   2. Stallion handlers

      a. Handling penis

      b. Handling mares

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APPENDIX E

SAMPLING MARE

A. Time to Sample

1. Best time: when mare is in estrus

   Reasons: a. organisms more likely to be found

   b. easier to enter dilated cervix

B. Samples to Collect

1. Either cervical or uterine swab

   Reason: fewer contaminating bacteria in cervix and particularly uterus

2. Clitoral fossa swab

   Reason: may be able to pick up chronic or carrier mare

3. Urethral swab

   Reason: same reason as for clitoral fossa

C. Equipment needed

1. Disposable sterile plastic vaginal speculum*
2. Tiegland swabs (modified)**
3. Pocket flashlight
4. Surgical gloves
5. Cotton
6. Disinfectant (chlorhexidine solution)***
7. Transport media (Stuarts or Aimes)
8. Scissors and pencil

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* Haver-Lockhart Laboratories - Shawnee, Kansas 66201

** Haver-Lockhart Laboratories - Shawnee, Kansas 66201

*** Nolvasan Solution - Fort Dodge Laboratories

D. Obtain History of Mare

1. Identification (name, tatoo no., age, etc.)
2. Breeding history
   a. stallion bred to
   b. presence of premature estrus
   c. presence of vaginal discharge, etc.

E. Technique

1. Restraint of Mare
   a. place mare either in breeding or examining chute or area
   b. may require tranquilizer
      1 - 100-200 mg xylazine*
      2 - other drugs could be used
2. Cleaning of external genitalia
   a. bandage tail and tie to side of mare
   b. thoroughly wash external genitalia with separate pledgets of cotton saturated with disinfectant**
   c. dry external genitalia with cotton
3. Passing vaginal speculum
   a. cover anterior part of speculum with light film of vaseline or lubricating jelly
   b. gently pass speculum into vagina. Apply gentle but firm pressure on speculum until it passes completely into vagina.

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* Rompum - Haver-Lockhart Laboratories

** Nolvasan Solution - Fort Dodge Laboratories

c. attach pocket flashlight to speculum and examine vagina for evidence of vaginitis or exudate

d. locate cervix and center speculum around external os of cervix

4. Preparation of Tiegland swab

a. while plastic speculum and swab are still within flexible cellophane container, cut end of container with scissors

b. shake out enclosed paper to use for identifying sample, mare, date of collection, etc.

c. without removing plastic speculum containing swab from cellophane container, remove red cap from end of plastic speculum

d. on "handle" end of white plastic rod within plastic speculum containing Tiegland swab, circle white plastic rod with pencil next to plastic speculum. This mark will be used for calculating distance Tiegland swab is passed through speculum and into cervix or uterus

e. carefully remove plastic speculum containing swab from cellophane container

5. Pass Tiegland swab into cervix or uterus

a. with the vaginal speculum centered on and surrounding the cervix, the Tiegland swab is quickly passed to the os of the cervix

b. with gentle manipulation the plastic speculum containing the swab is gently passed into

the cervix a distance of 1-3 inches. (do not force speculum through the cervix if it does not glide through easily)

c. the inner white plastic rod containing the swab is gently forced a distance of 1-3 inches into the cervix or uterus watching the pencil mark for depth of passage

d. rotate the swab several times and then retract it back into the plastic speculum so that the cotton swab is completely covered (use pencil mark for guide)

e. withdraw the plastic speculum and swab from the vaginal speculum

f. open end of cellophane container and replace plastic speculum and swab into original position

g. locate the 2 circular marks circumventing the plastic speculum and decide where to break the speculum and swab. (usually the second mark is allowing for the longest swab)

h. break the speculum on the mark and withdraw portions of plastic speculum and plastic swab holder and discard

i. with cotton swab contained within the remaining plastic speculum, replace red caps on either end of speculum while still in cellophane container

j. tie knot on cellophane bag next to speculum so as not to lose swab

k. replace paper with identification into cellophane container

l. tie knot on end of cellophane bag

6. Passing swab into clitoral fossa

a. clitoral fossa is located on floor of vagina just anterior to ventral commisure of vulva

b. by spreading lips of the vulva with the left hand clitoral fossa is exposed for easy culturing

7. Passing swab into urethra

a. again locate urethra with gloved finger on floor of vagina about 6 inches anterior to the clitoral fossa

b. pass swab 1-2 inches into urethra and remove

c. prepare swab similar to above

Handling swabs for delivery to diagnostic laboratory

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